Limb Development

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Ed Laufer elaufer@columbia.edu

What we will cover...

QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.

What signals control initiation of limb bud formation ?

What signals transform the *embryonic limb bud* into a *mature limb* comprised of a precisely interconnected array of many different tissues?

The developing limb is a model environment to study development

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

QuickTime™ and a
Photo - JPEG decompressor
are needed to see this picture.

cell fate specification - eg bone vs muscle vs tendon

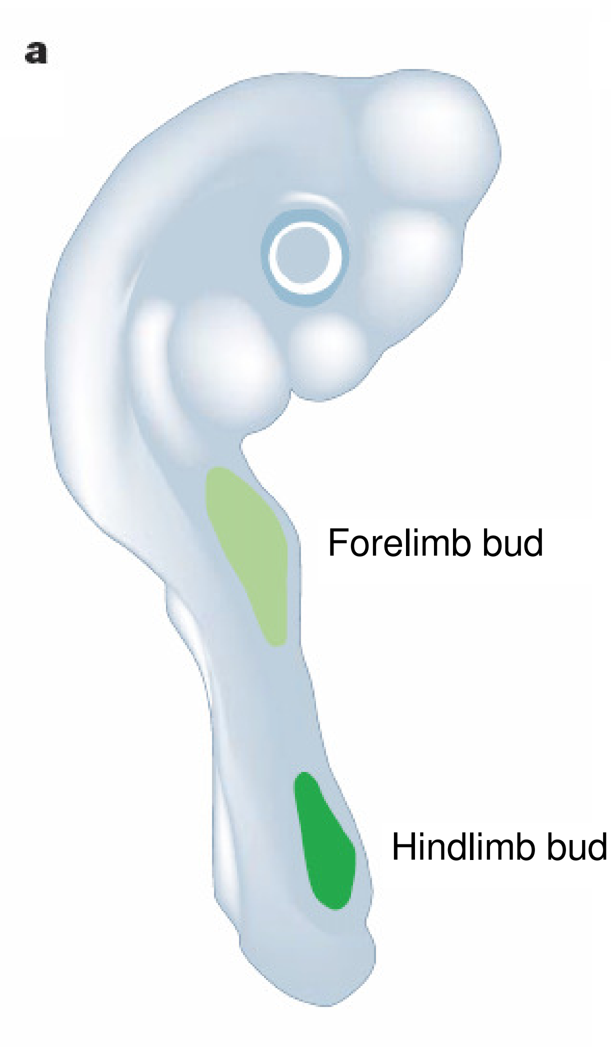
cell migration and pathfinding - muscle precursors, innervation

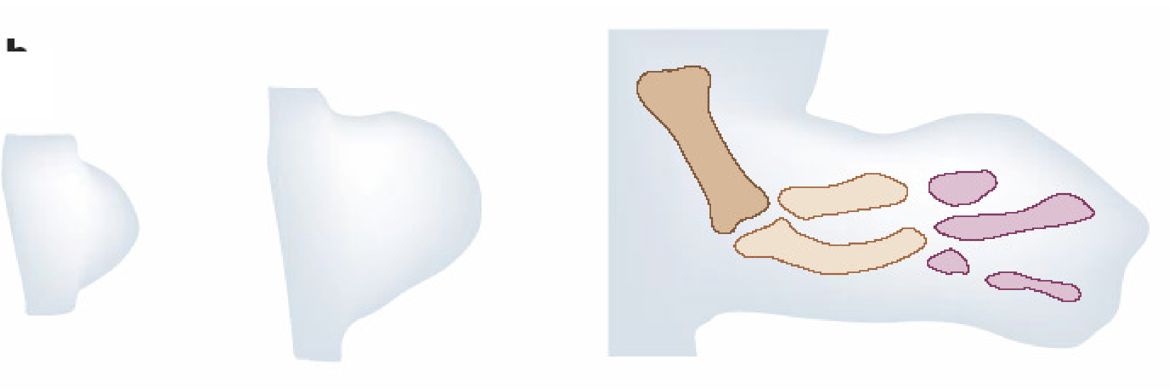
cell proliferation/programmed cell death (apoptosis)
- correct tissue morphology, interdigital cell death

tissue patterning - organization of structures in 3D space

limb abnormalities are associatedwith many human congenit

EARLY LIMB PATTERNING:

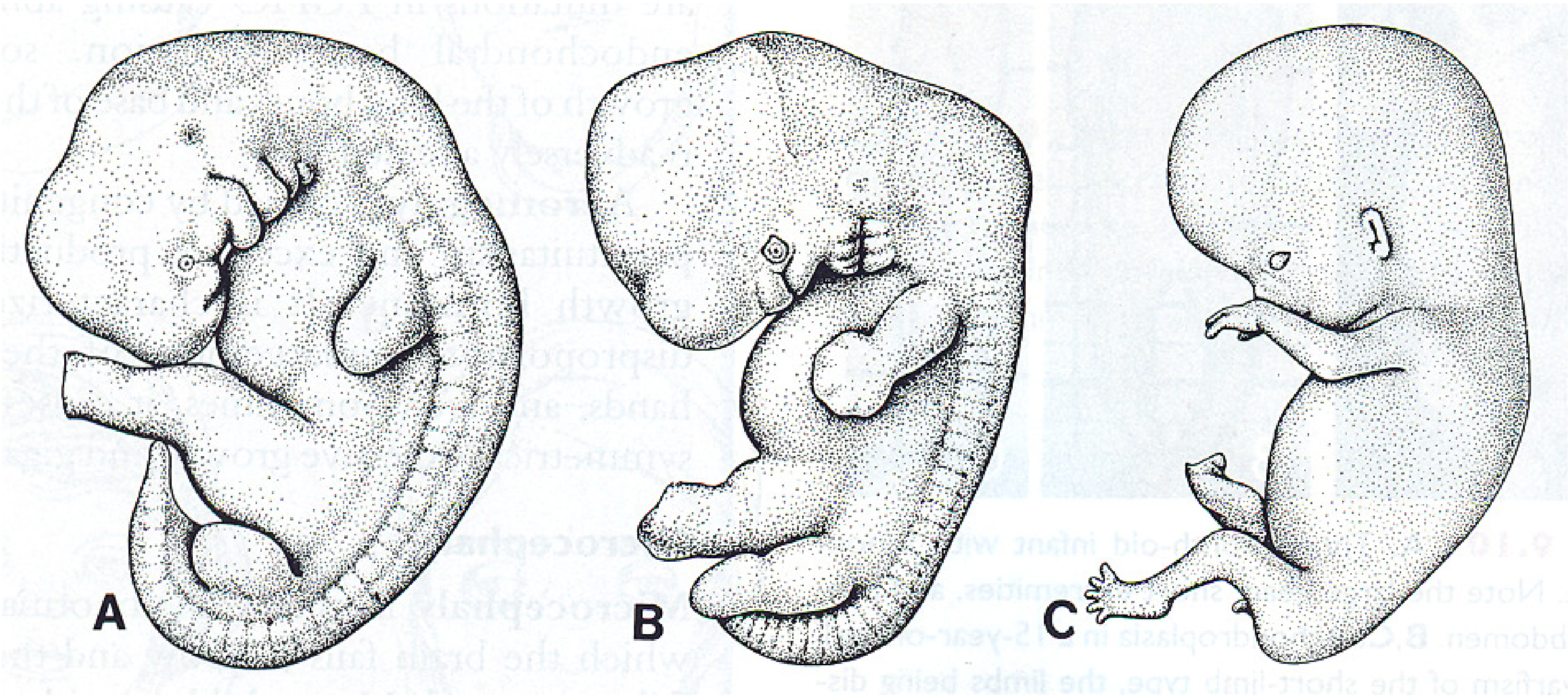




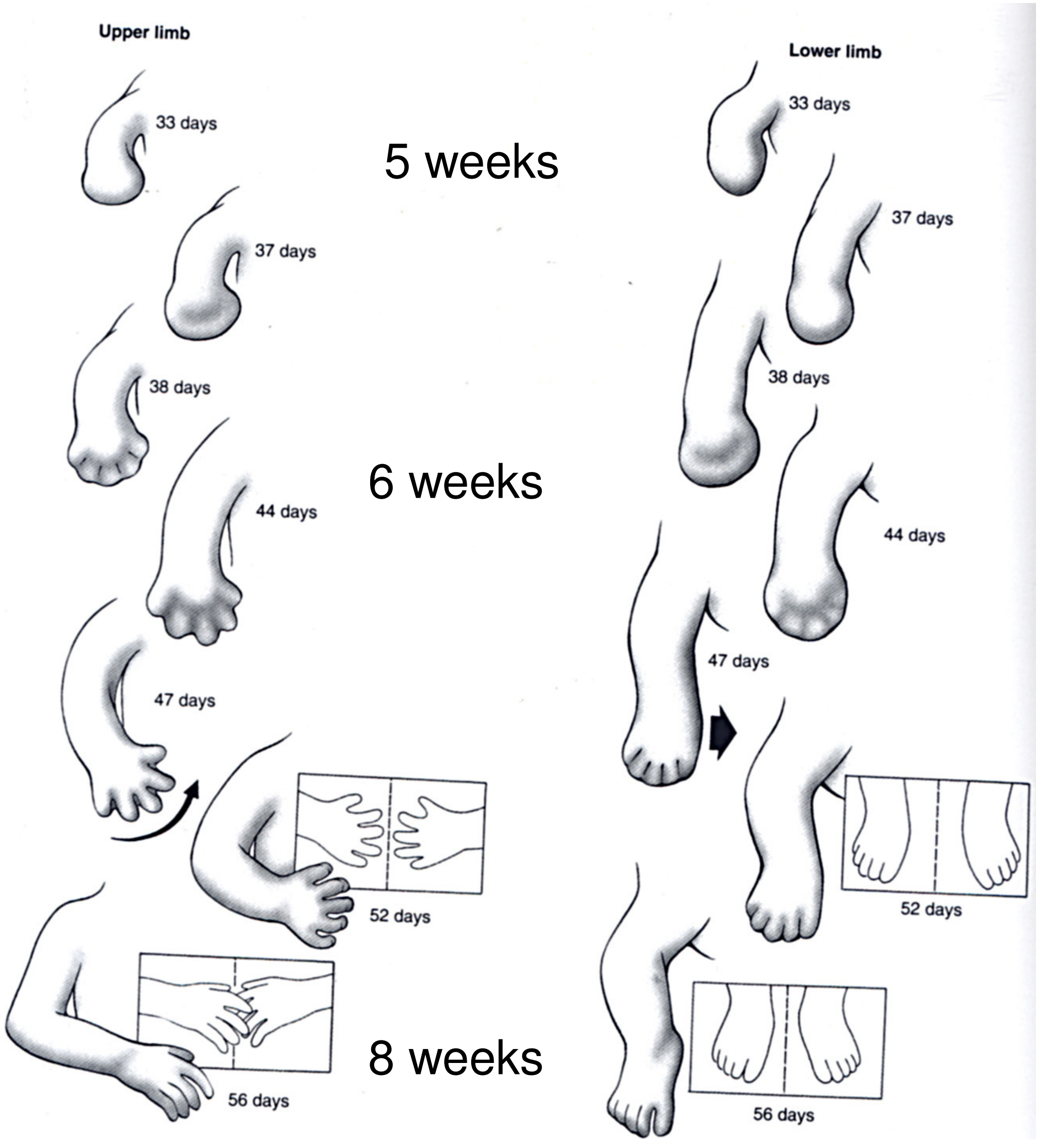
Limb formation initiates during the fourth week of development (E9.0 in mouse) as the primary axis (AP) is still elongating. First the forelimb and then the hindlimb begin as protrusions from the lateral plate mesoderm at the sides of the embryo. Limb buds consist of a core of mesenchyme and an outer covering of ectoderm.

1 in 200 live human births display limb defects.

Human Limb Development



5 weeks 6 weeks 8 weeks



A-P (fingers); D-V (palm); P-D (length)

Limbs rotate inward

Day 33: hand plate, forearm, shoulder

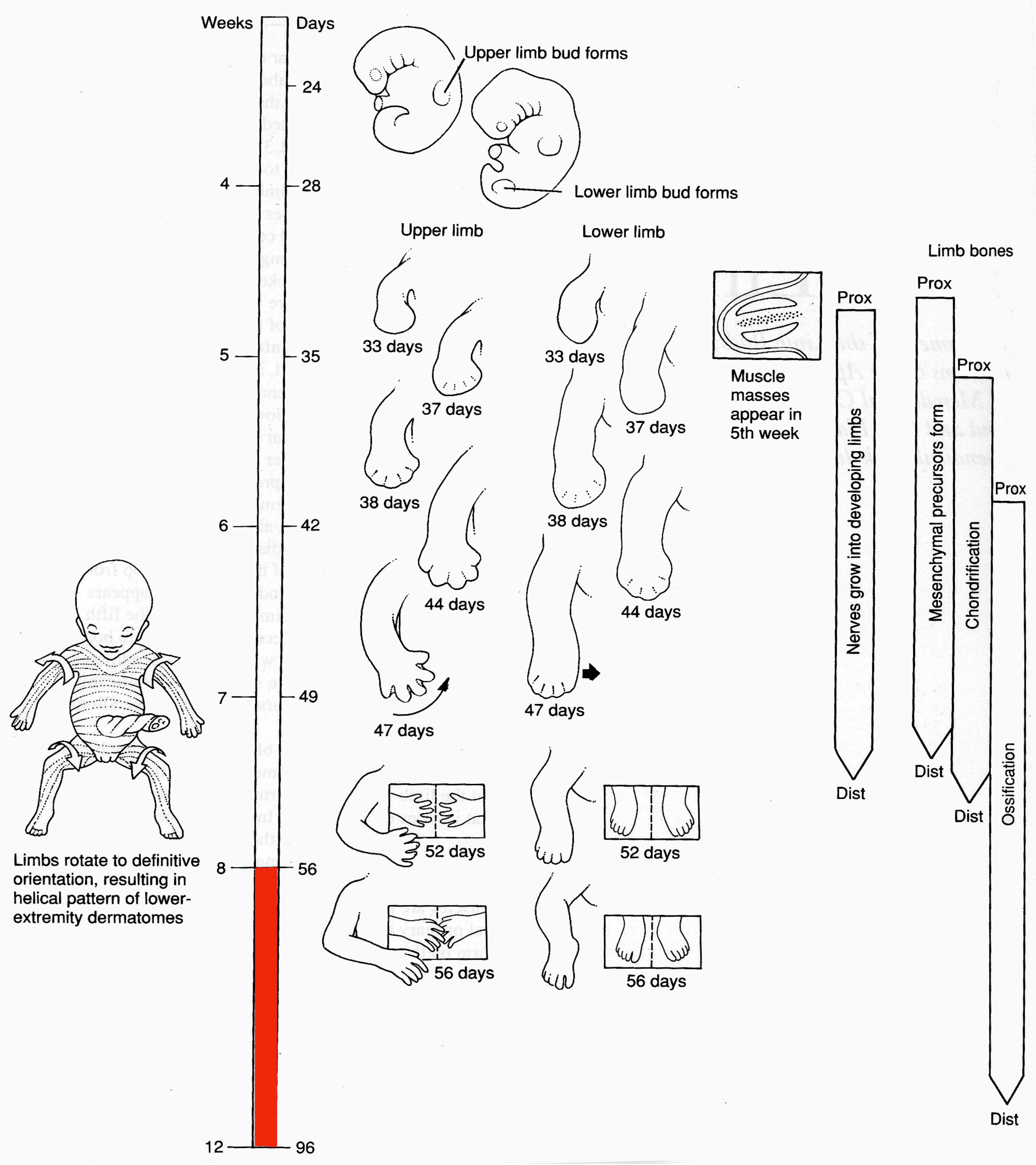
Day 37: Carpal region, digital plate

Day 38: Finger rays, necrotic zones

Day 44: toe rays

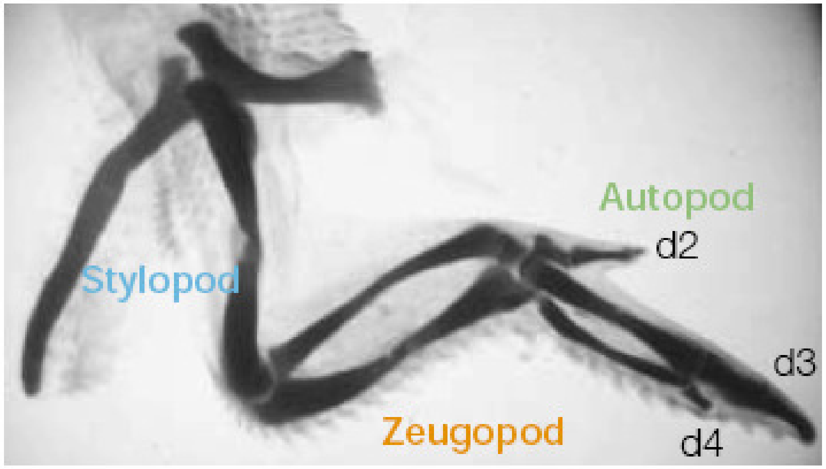
Day 47: horizontal flexion

Day 52: tactile pads

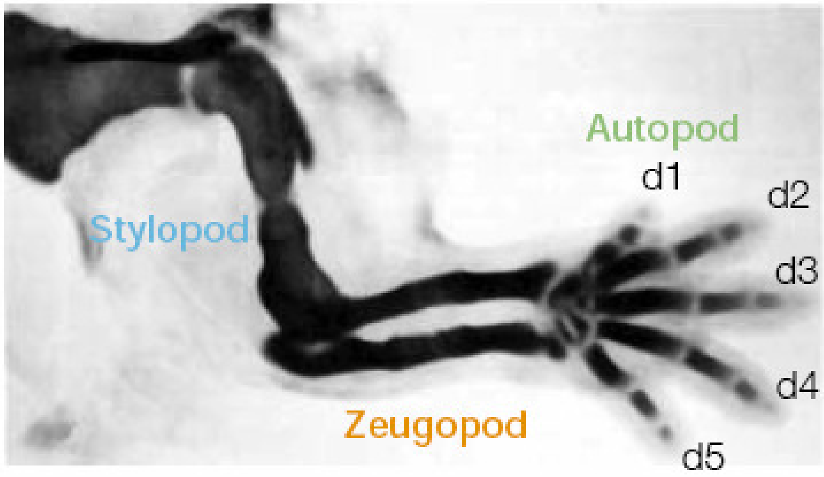

Weeks
Days
24
4
28
5
35
6
42
7
49
8
56
12
96
Upper limb bud forms
Lower limb bud forms
Upper limb
Lower limb
33 days
37 days
38 days
44 days
47 days
33 days
37 days
38 days
44 days
47 days
52 days
56 days
52 days
56 days
Muscle masses appear in 5th week
Prox
Nerves grow into developing limbs
Dist
Limb bones
Prox
Mesenchymal precursors form
Dist
Prox
Chondrification
Dist
Prox
Ossification
Dist
Limbs rotate to definitive orientation, resulting in helical pattern of lower-extremity dermatomes

Limb skeletal elements:

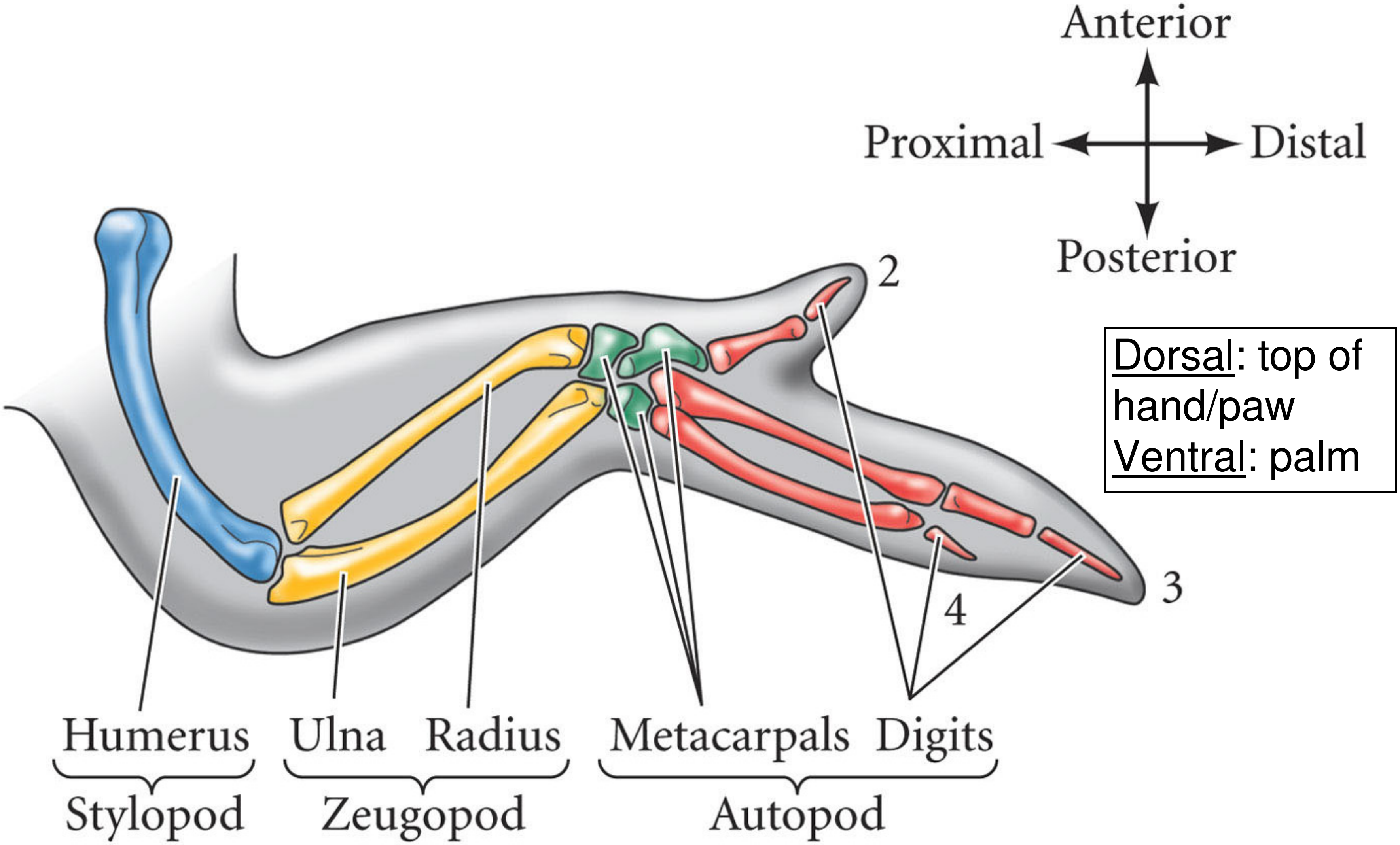
Chicken



Mouse



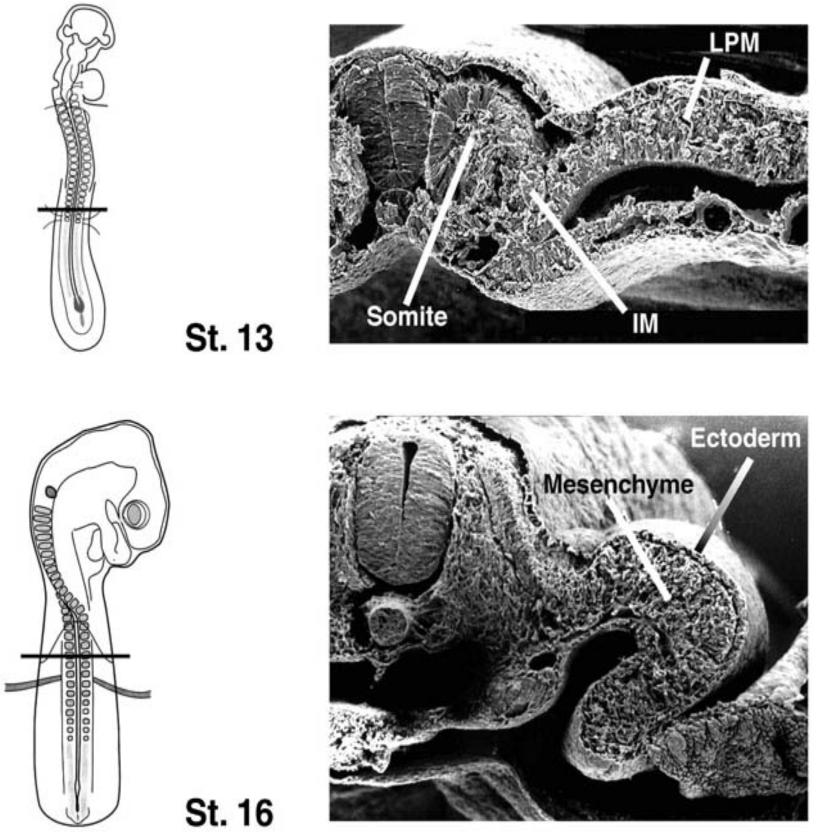
Stylopod: The proximal element of a limb.
The humerus in the forelimb and femur in the hindlimb
Zeugopod:The intermediate element of a limb.
The radius and ulna in the forelimb and the tibia and fibula in the hindlimb
Autopod: The distal elements of a limb.
The wrist and the fingers in the forelimb and the ankle and toes in the hindlimb



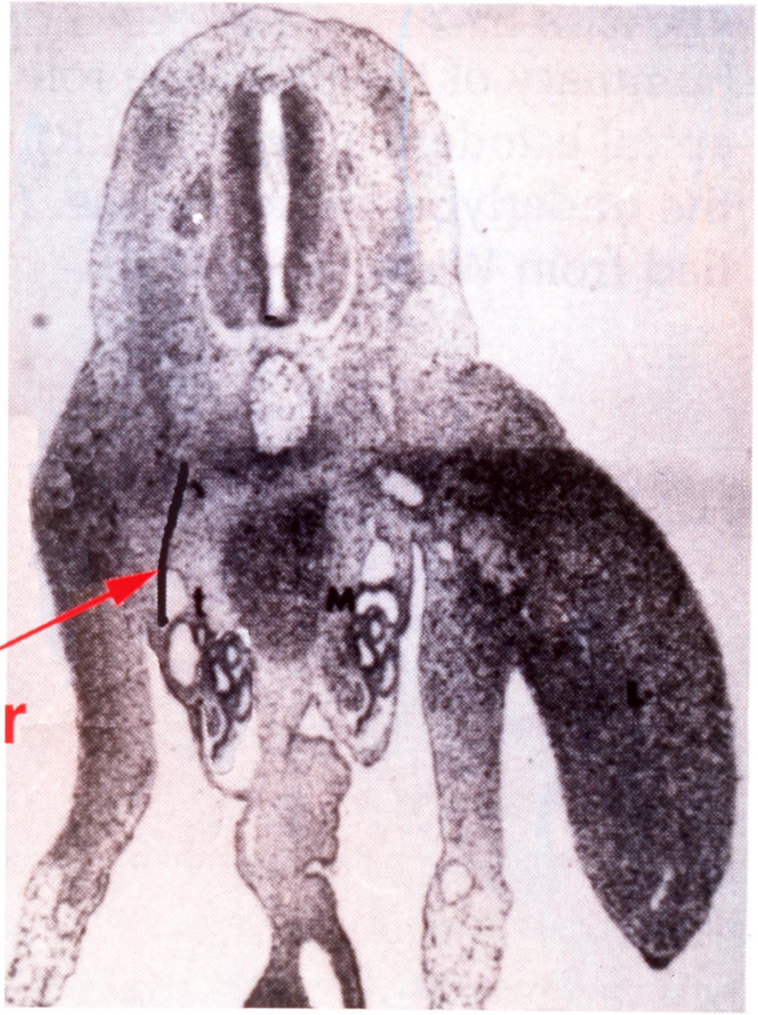
Dorsal: top of hand/paw
Ventral: palm

DEVELOPMENTAL BIOLOGY, Eighth Edition, **Figure 16.1** © 2006 Sinauer Associates, Inc.

How is limb initiation controlled?

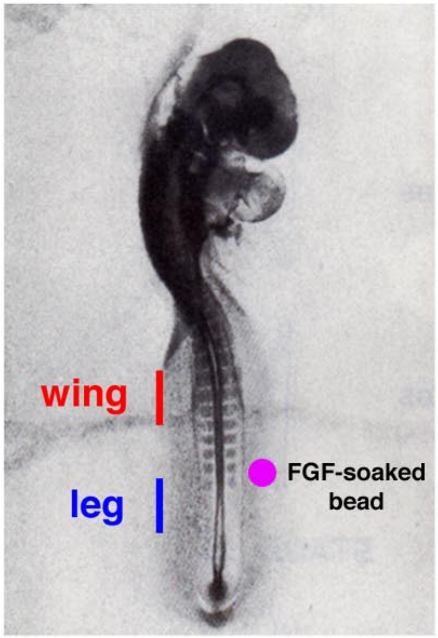


Barrier between the intermediate and lateral plate mesoderm prevents limb bud formation

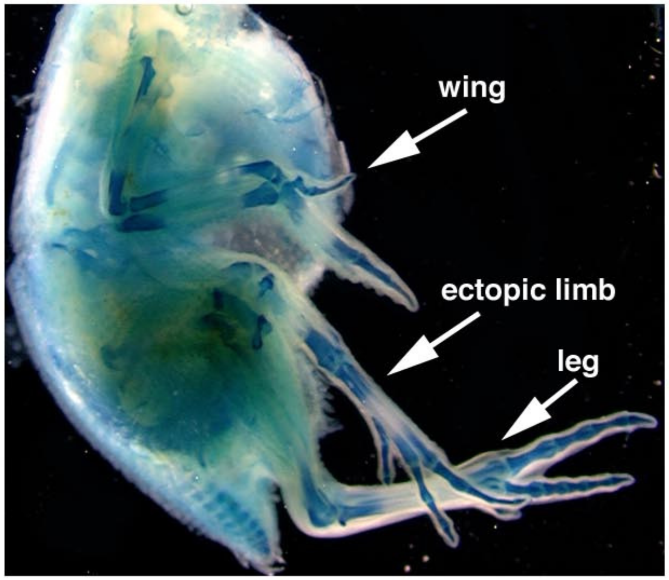


Foil Barrier

Secreted signaling molecules, fibroblast growth factors (FGFs) can induce ectopic limb formation from cells in the flank of the chick embryo



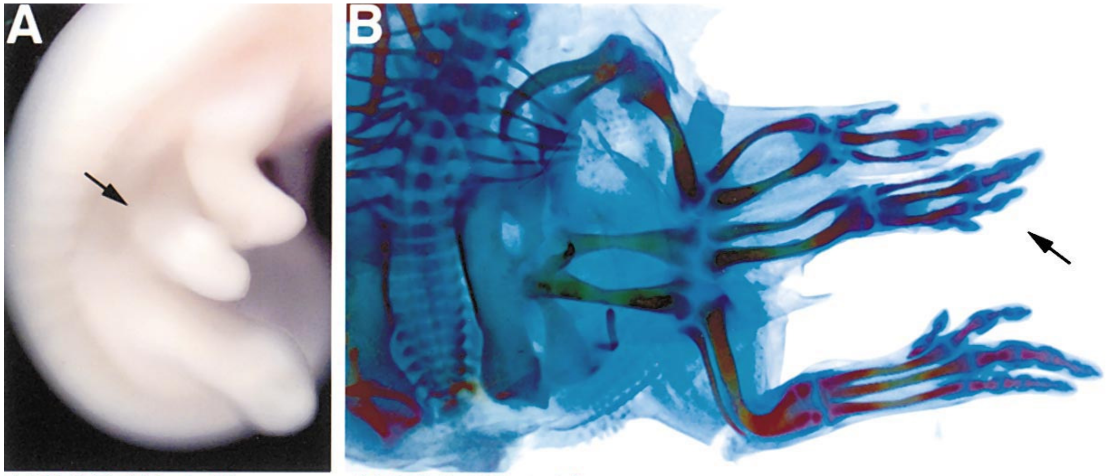
Pre-limb bud stage
chick embryo(H+H st15)



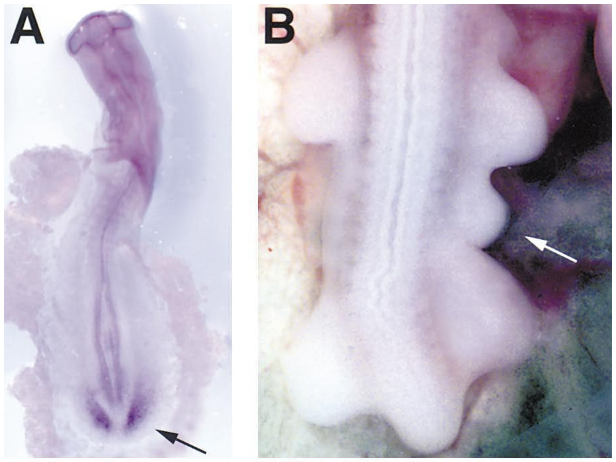
After 9 further days of incubation (st36)

-a single molecule (FGF) is capable of inducing cascade leading to limb formation
-cells in the flank are competent to respond to this secreted signal

Members of another family of secreted proteins, Wnts, can also induce formation of ectopic limbs from the inter-limb lateral plate mesoderm



RCAS-Wnt-2b *RCAS-Wnt-2b*

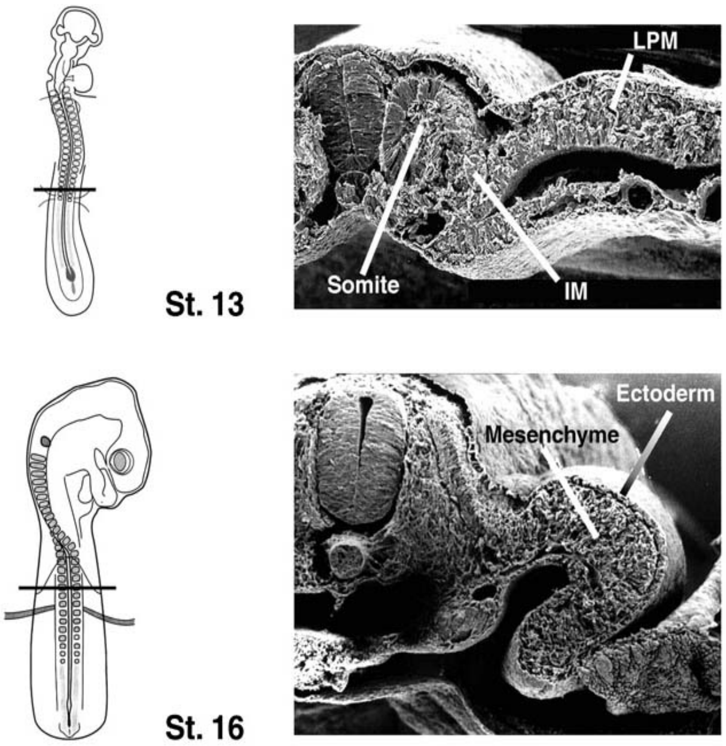


Wnt-8c
St.11/12HH

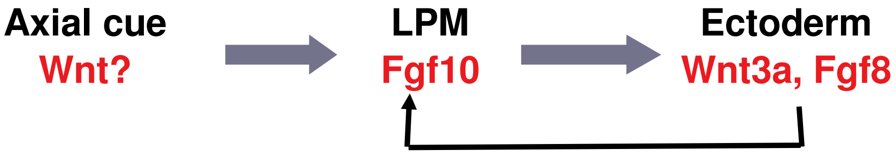
RCAS-Wnt-8c

Kawakami et al., Cell 2001

Limb initiation



lpm: lateral plate mesoderm



Axial cue
Wnt?

LPM
Fgf10

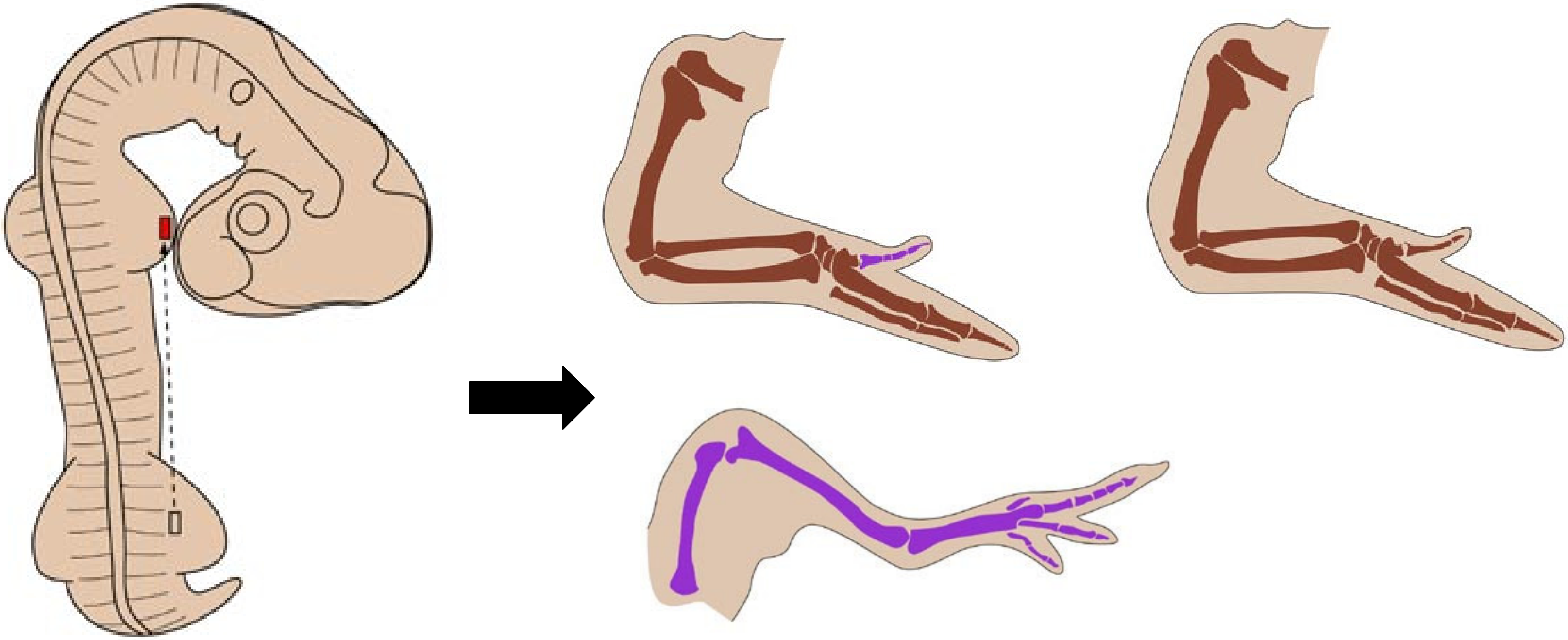
Ectoderm
Wnt3a, Fgf8

Transfers information from the body to the limb axis

Limb-type specification

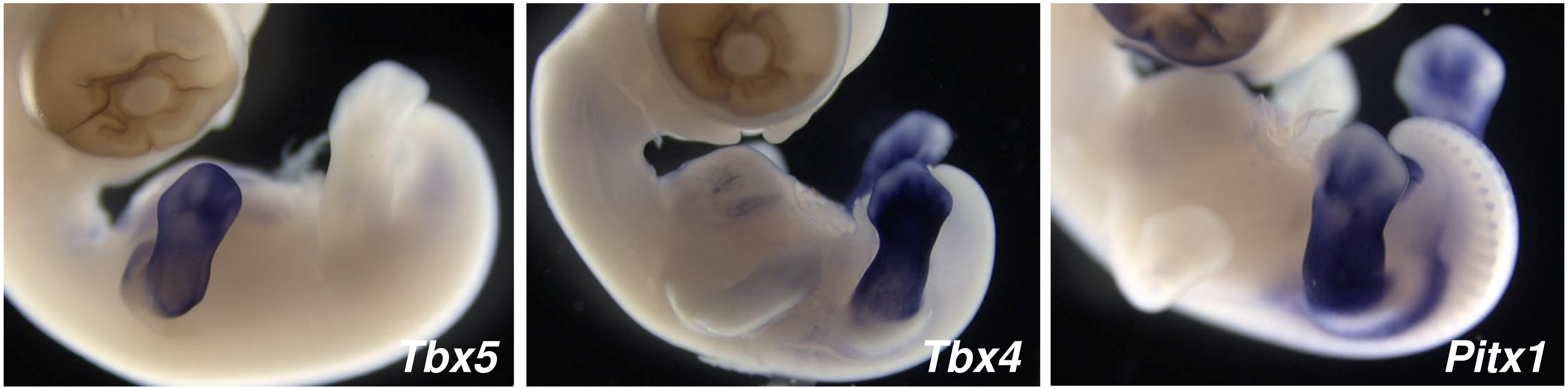
Morphologically uniform limb buds develop to form morphologically distinct limb elements

Cell identity and plasticity in the developing limb bud



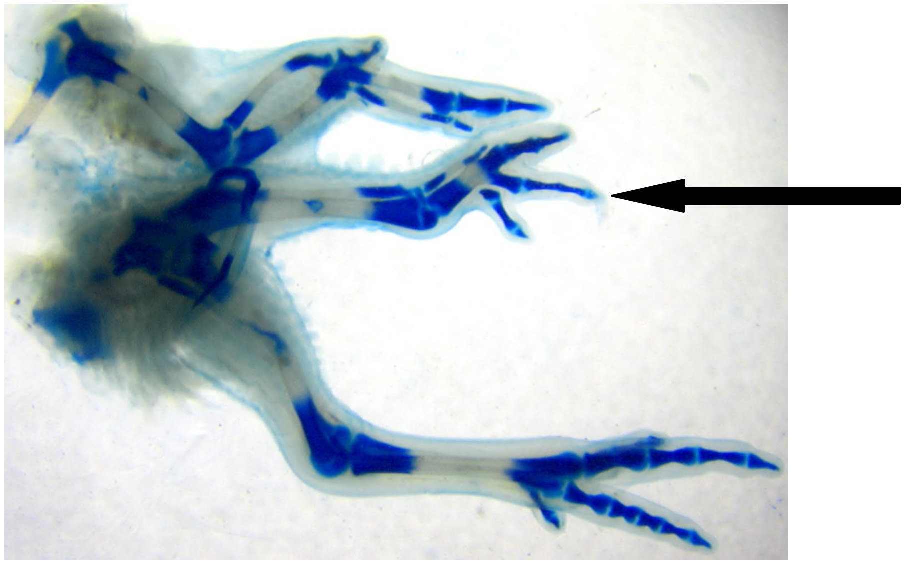
Saunders et al., 1957, 1959

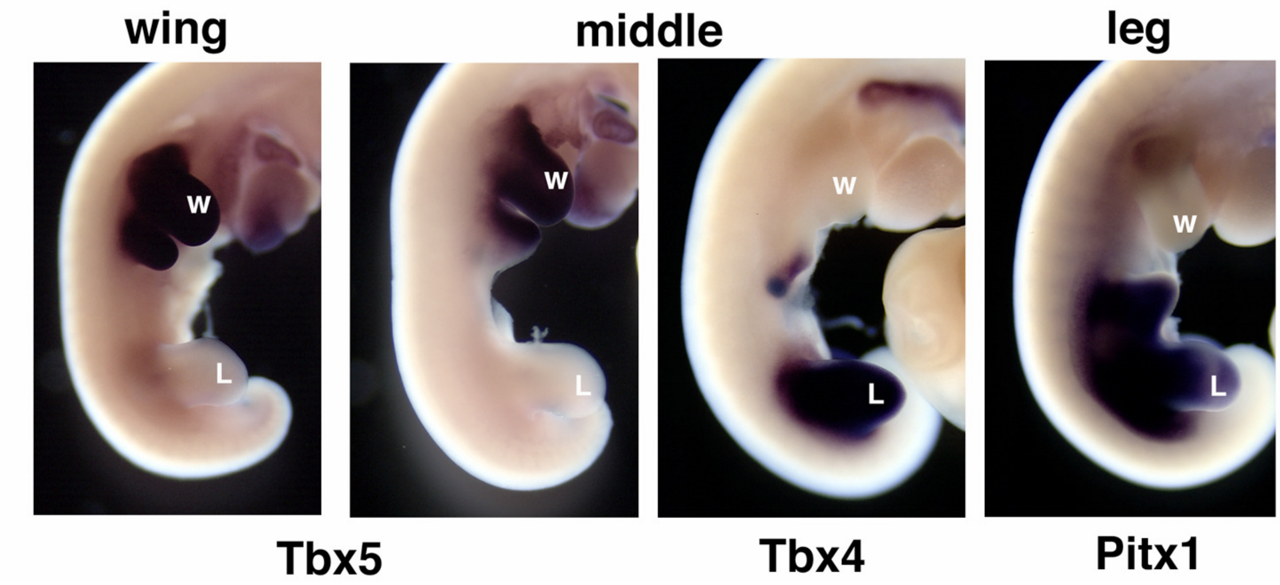
Limb-type specification: candidate genes



chick st.29

A source of FGF applied to the interlimb flank induces ectopic limb formation in the chick embryo: Tbx expression correlates with morphology





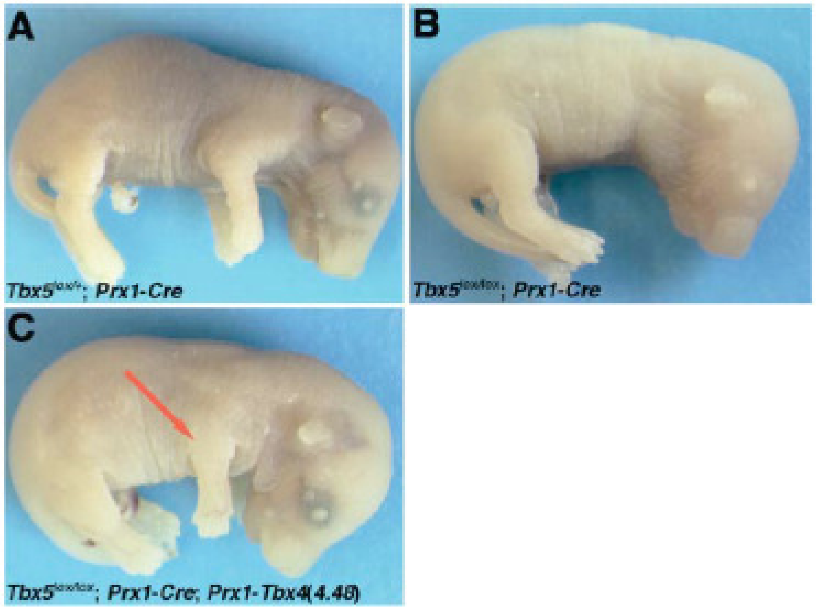
Conditional knock-out of *Tbx5* in the limbs leads to the absence of the forelimbs

$Tbx5^{lox/lox}$*;Prx1cre*

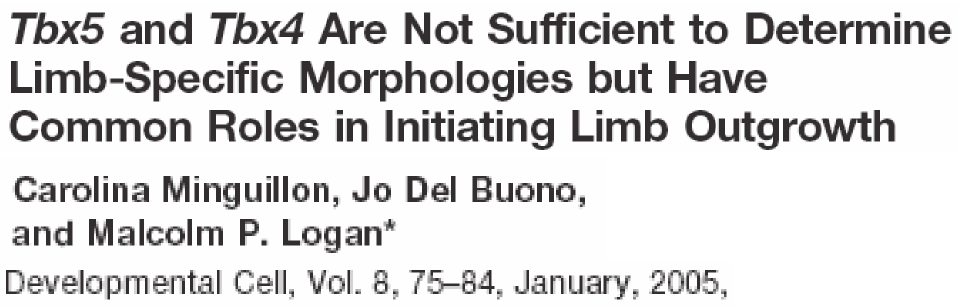
Replacement of Tbx5 with Tbx4 in the limb rescues limb outgrowth. The limb remains a forelimb.

Control

no Tbx5 in limb



Tbx4 for Tbx5: Rescue



***Tbx5* and *Tbx4* Are Not Sufficient to Determine Limb-Specific Morphologies but Have Common Roles in Initiating Limb Outgrowth**

Carolina Minguillon, Jo Del Buono,
and Malcolm P. Logan*

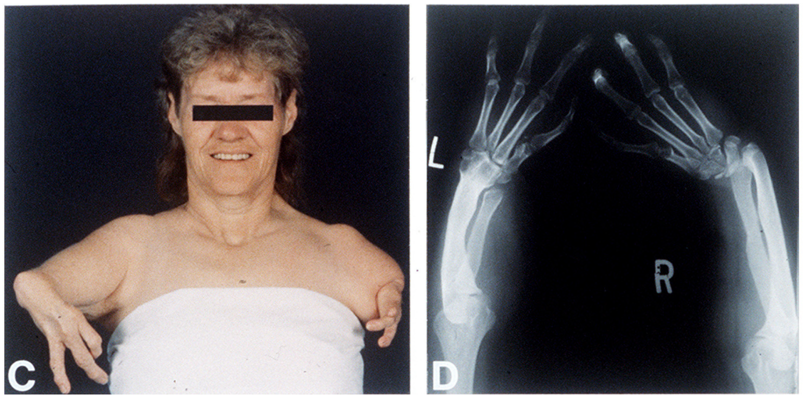
Developmental Cell, Vol. 8, 75–84, January, 2005,

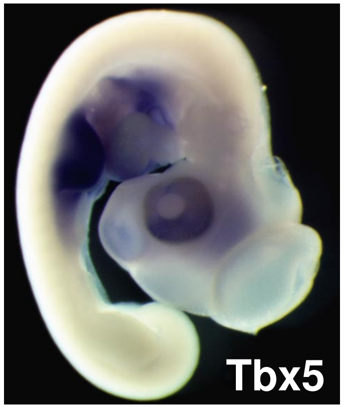
Correlation is not causality: causality must be determined experimentally

Limb abnormalities are associated with many human congenital syndromes

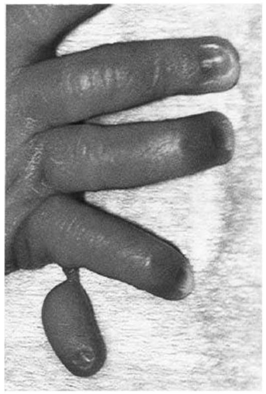
second most-common congenital abnormality in human live births
also common abnormality following environmental insult (eg Thalidomide)

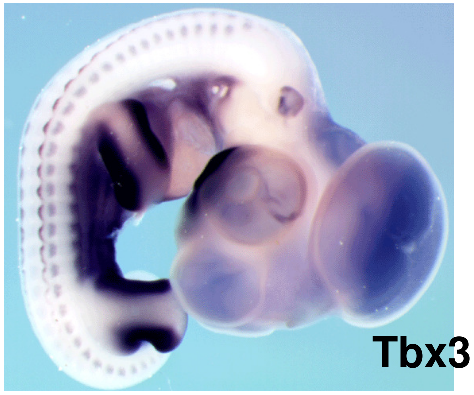
Mutations in Human *TBX5* are associated with Holt-Oram Syndrome (HOS)





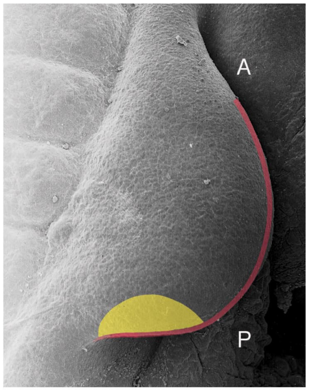
Mutations in human *TBX3* are associated with Ulnar-Mammary Syndrome (UMS)

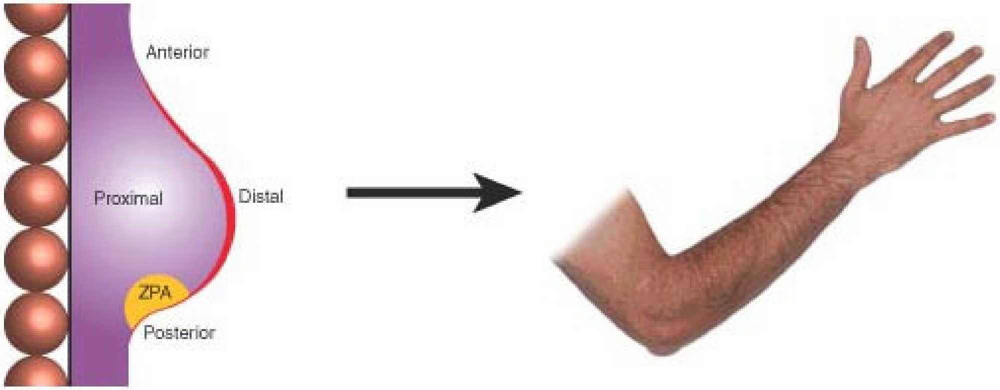


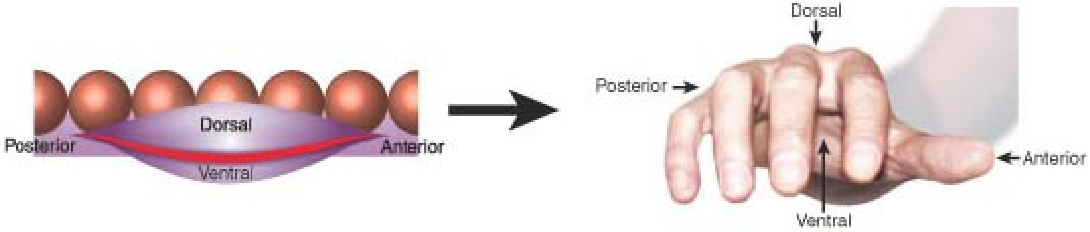


3 signaling centers pattern the 3 primary axes of the limb

Apical ectodermal ridge (AER) - proximal-distal
Zone of polarizing activity (ZPA) - anterior-posterior
Dorsal ectoderm - dorsal-ventral

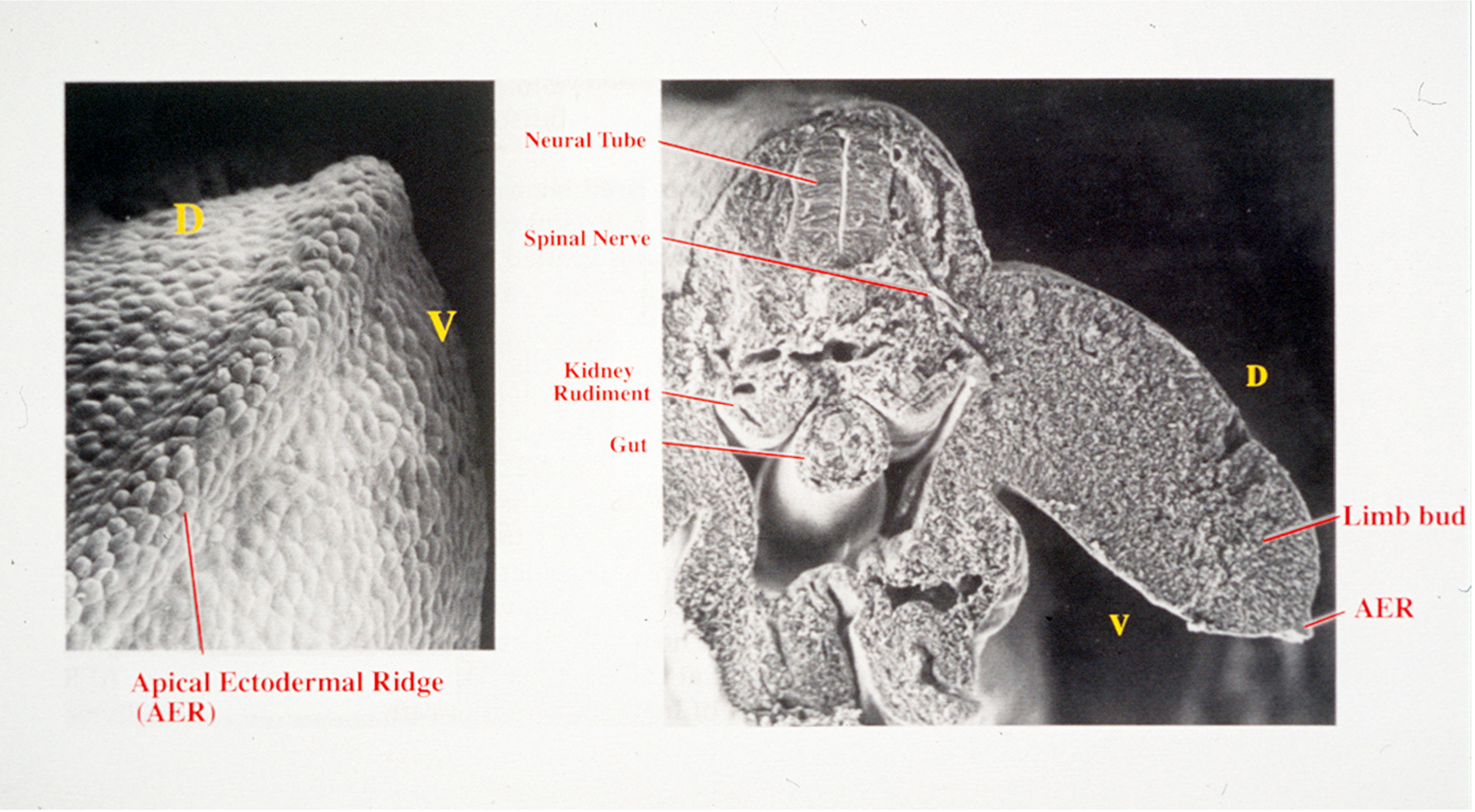




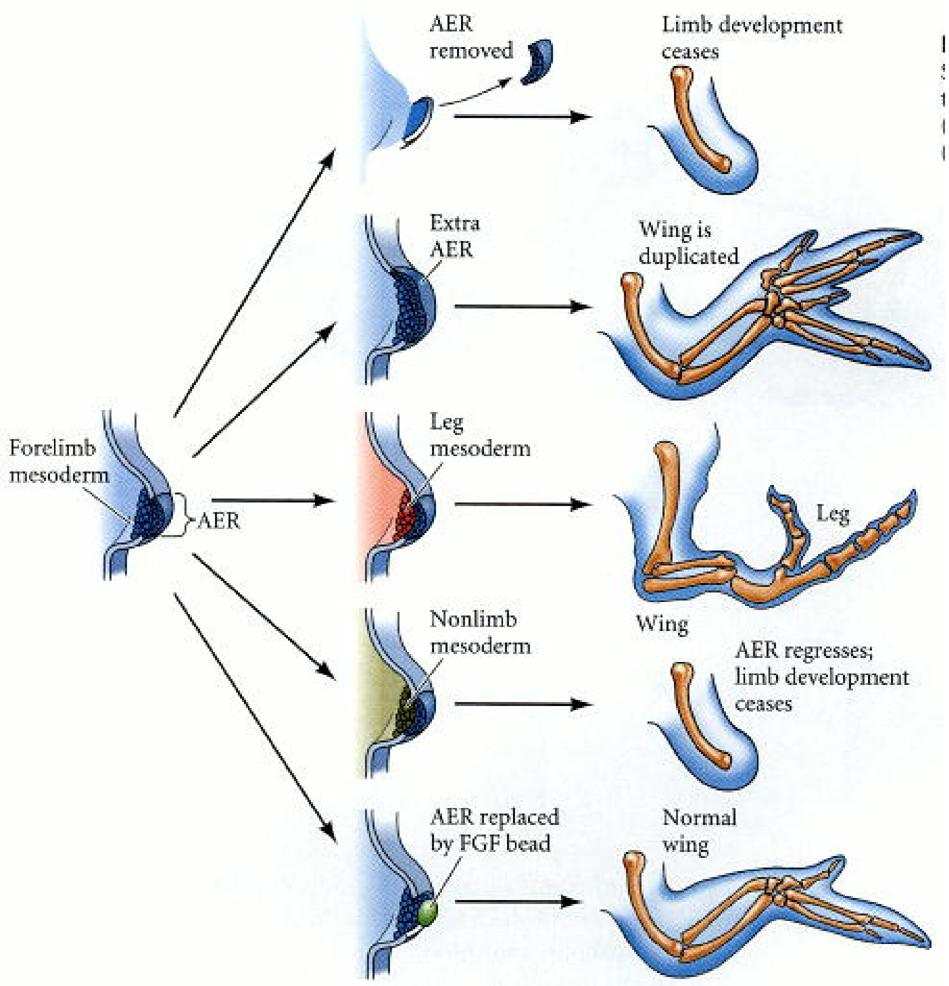


Apical ectodermal ridge (AER) - proximal-distal axis

Signals from the AER maintain limb outgrowth



AER manipulations give insight into AER function



required

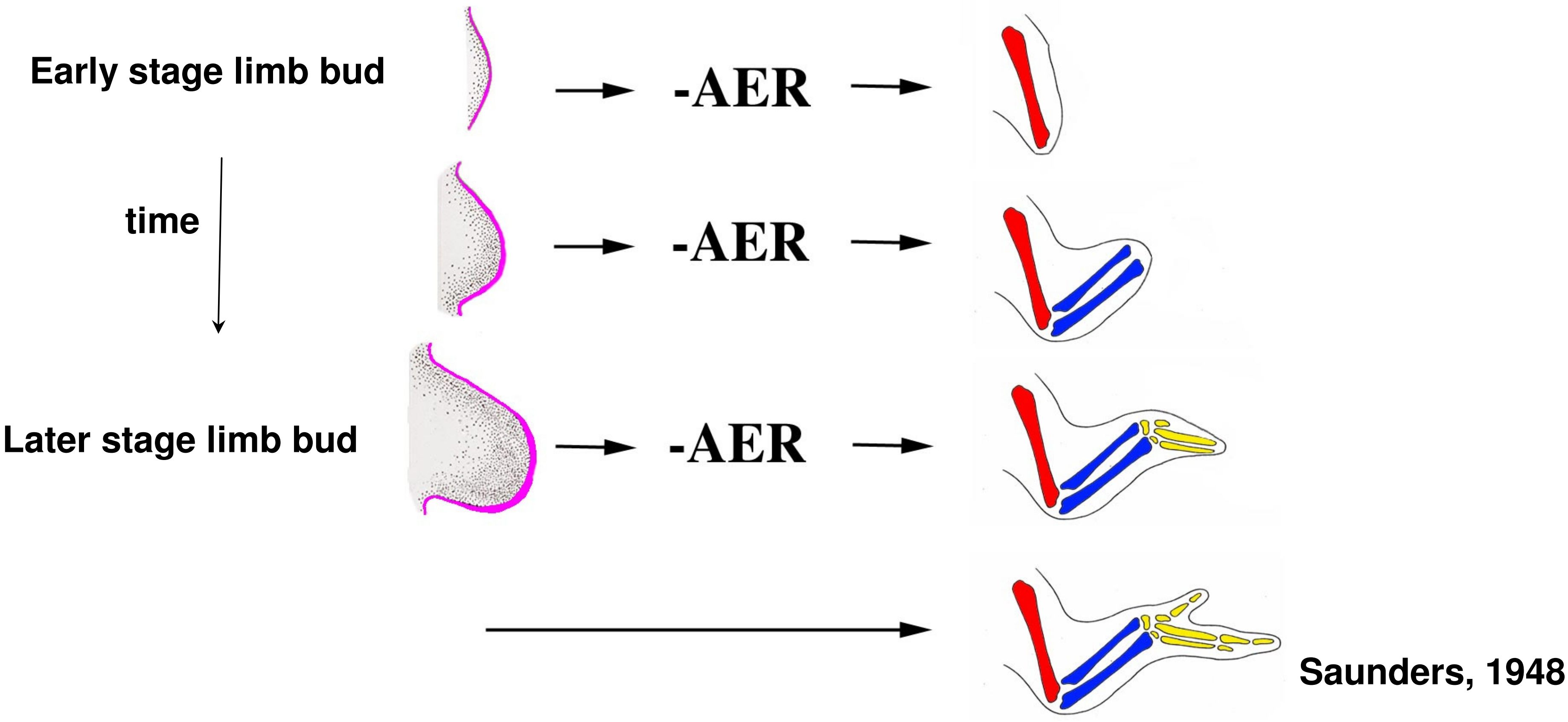
promotes outgrowth

not instructive

not instructive

FGF source

Conclusions from ‘classical’ embryological studies
- the AER is required for normal proximal-distal pattern



Early stage limb bud

time

Later stage limb bud

-AER

-AER

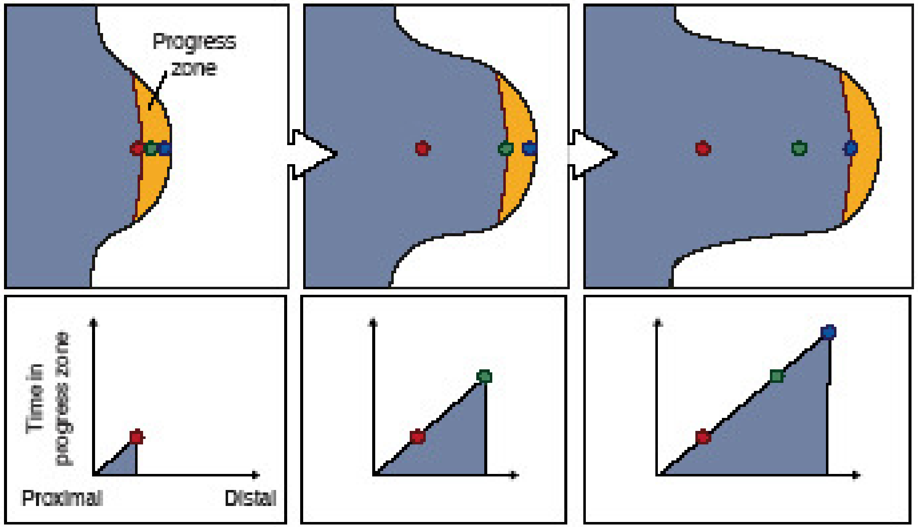
-AER

Saunders, 1948

- required for outgrowth, full P-D pattern
- elements laid down in proximal to distal progression

The progress zone (PZ) model

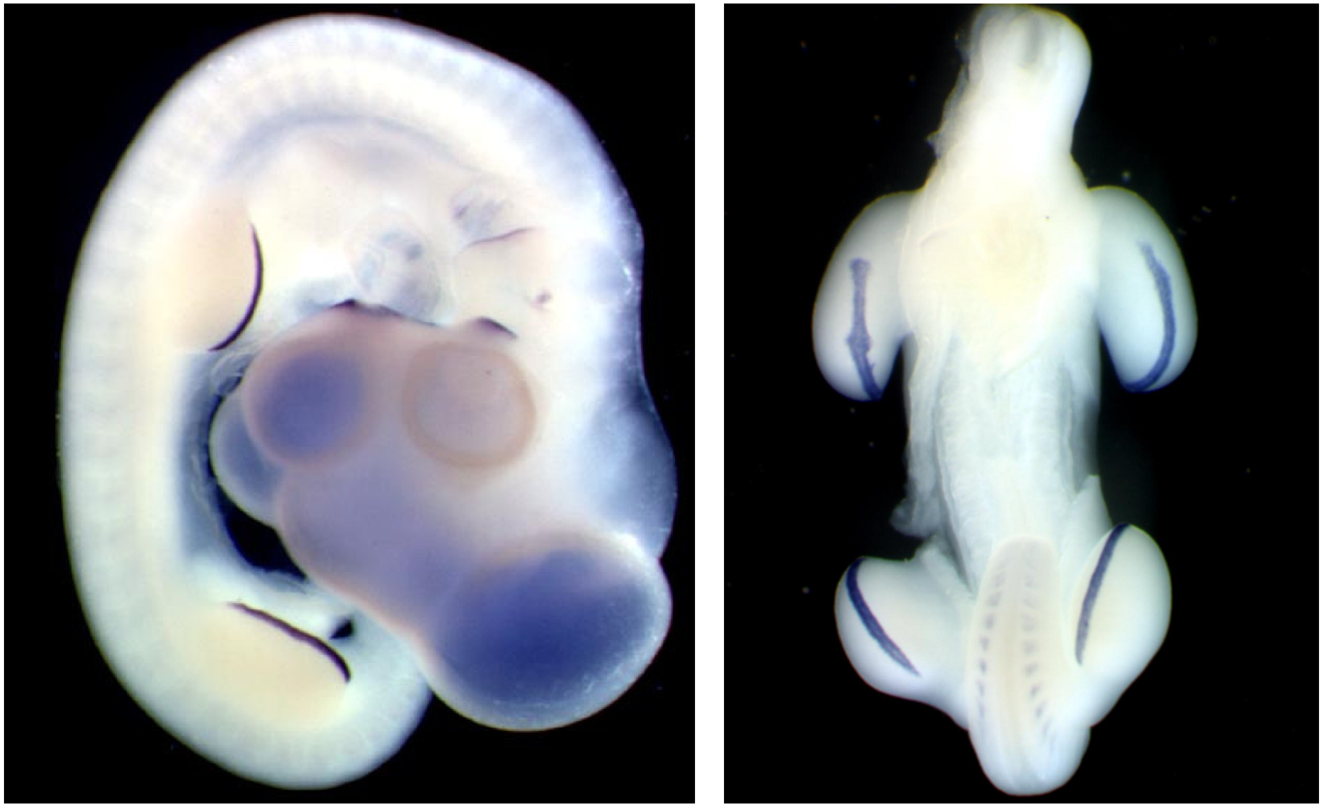
Summerbell et al. Nature 1973



The length of time a cell spends in the PZ may determine proximal-distal identity

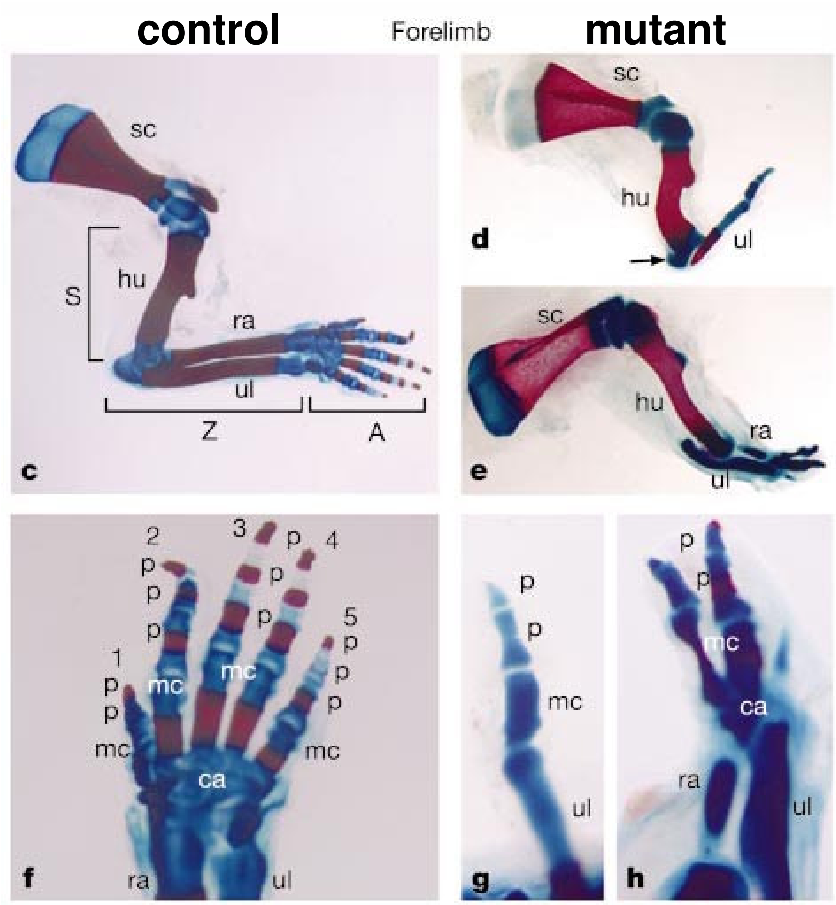
What factors are regulating outgrowth?

Evidence supporting a role for FGFs in proximal-distal patterning
- Fgf8 is expressed in the apical ectodermal ridge (AER)
i.e. present at right time, in right place



Whole mount RNA *in situ* hybridization - chick (st23)

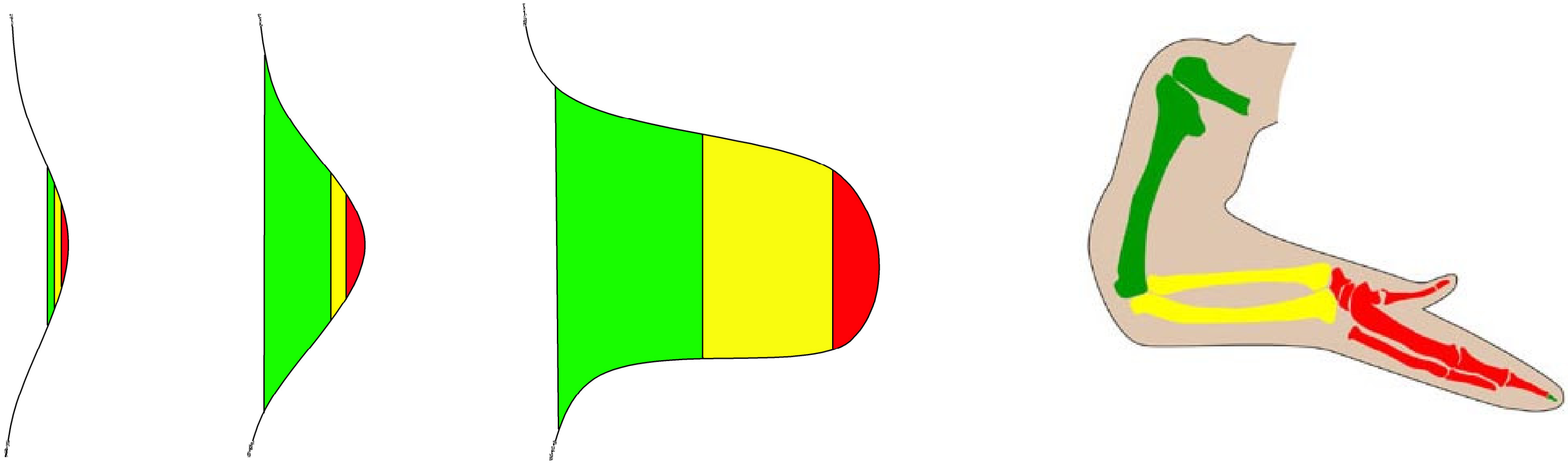
Fgf8/Fgf4 double knock-out mouse: a genetic equivalent to AER removal in the chick



Sun et al., Nature 2002

Limb outgrowth disrupted but distal structures do form. This would not be predicted by the progress zone model

Alternative model: early allocation followed by expansion



progenitor pools are specified early during limb outgrowth

A signal (FGF) from the AER progressively expands these preexisting populations

AER defects give rise to proximodistal outgrowth phenotypes

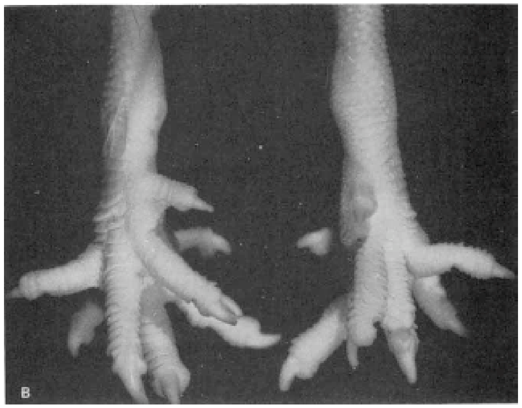
Split-hand/split-foot malformation (SHFM) caused by p63 mutation: reduced AER maintenance

Am J Hum Genet. 2000 July; 67(1): 59–66.
Published online 2000 June 5.

Pediatric Radiology 2003
10.1007/s00247-003-1017-3

Diplopodia: ectopic AER?

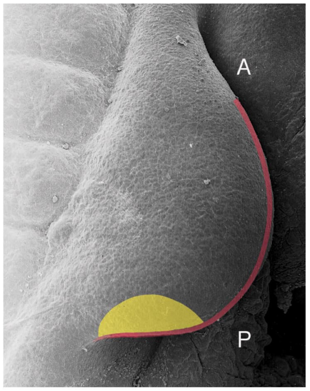
Eudiplopodia: ectopic AER (chicken)

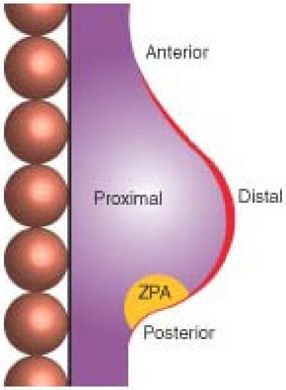


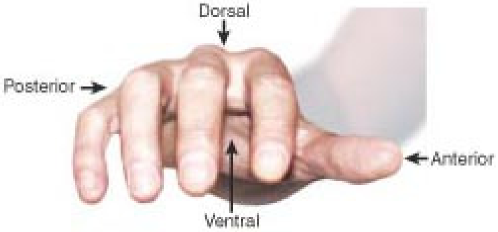
J. Exp. Zool. 17n. 219-230.

The axes of the limb

Zone of polarizing activity (ZPA) - anterior-posterior

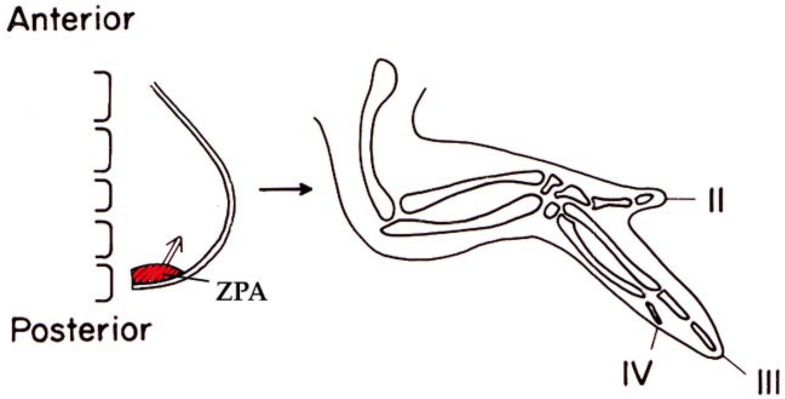




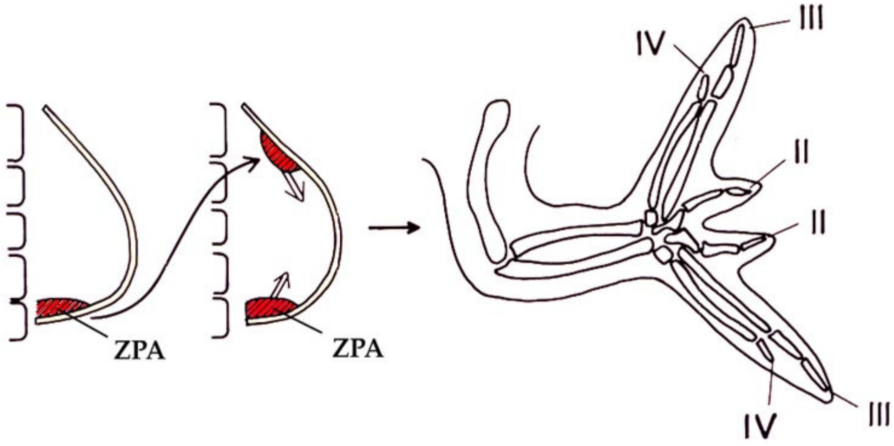


A region of cells in the posterior limb bud, the zone of polarizing activity (ZPA) Is important for patterning the anterior-posterior axis of the limb

Again, an important signaling center in the limb was initially identified in ‘classical’ embryological experiments

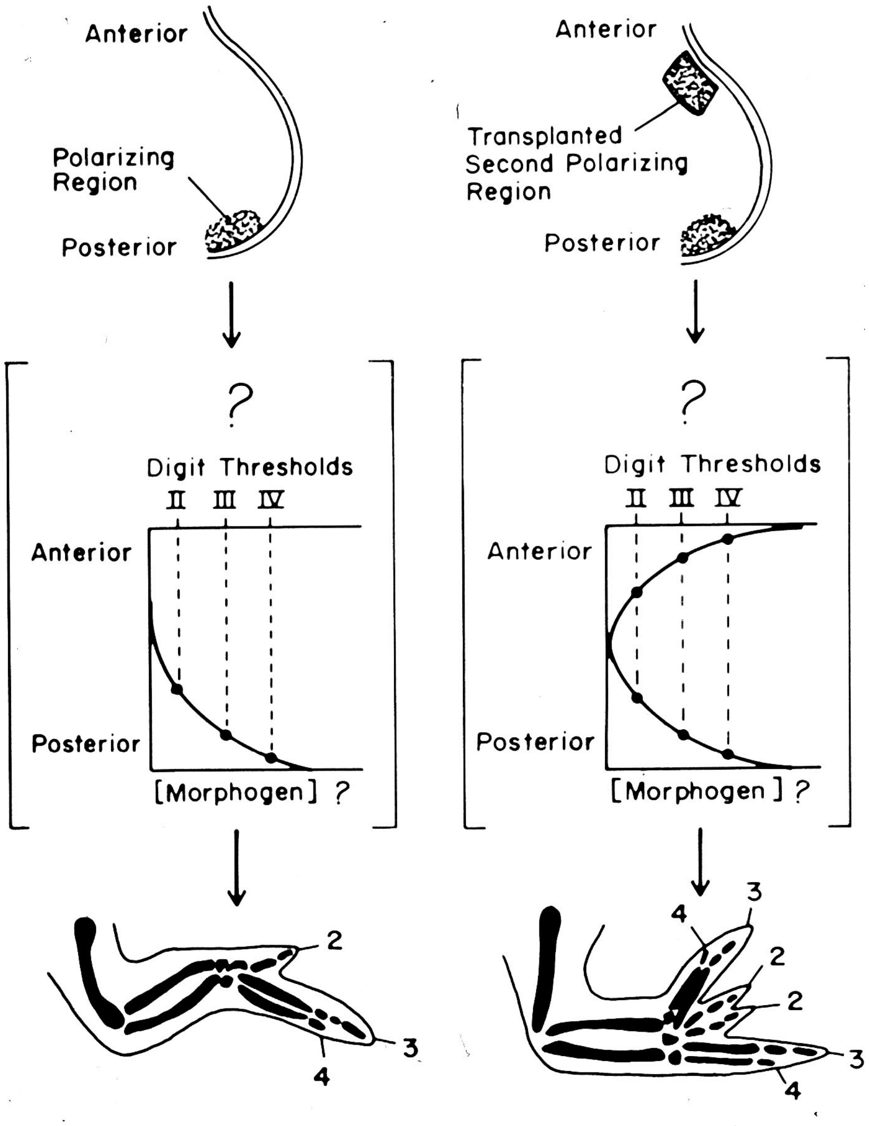


Ectopic digits are not derived from the ZPA graft itself. They are induced in the host tissue. This is a non cell-autonomous phenotype.



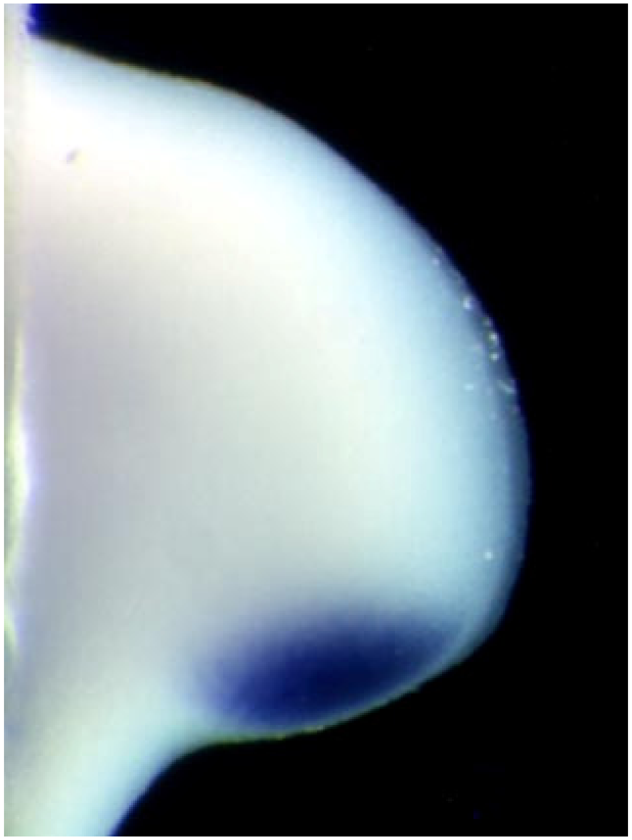
Saunders and Gasseling 1968

Morphogen model: cell identity via threshold responses to a gradient of signaling molecule



circa 1969

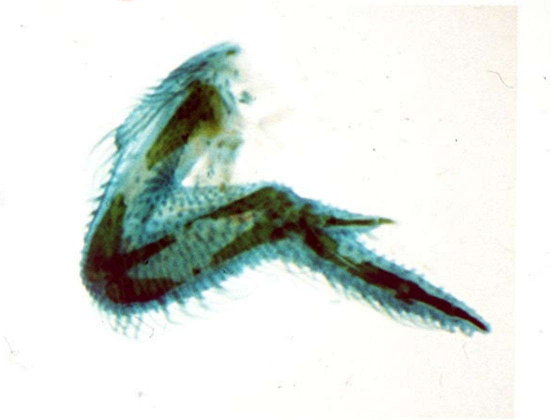
Sonic Hedgehog (*Shh*), a vertebrate homolog of the Drosophila (fruit fly) gene *hedgehog*, is expressed in the ZPA



Direct evidence for a role of Shh in anterior-posterior patterning

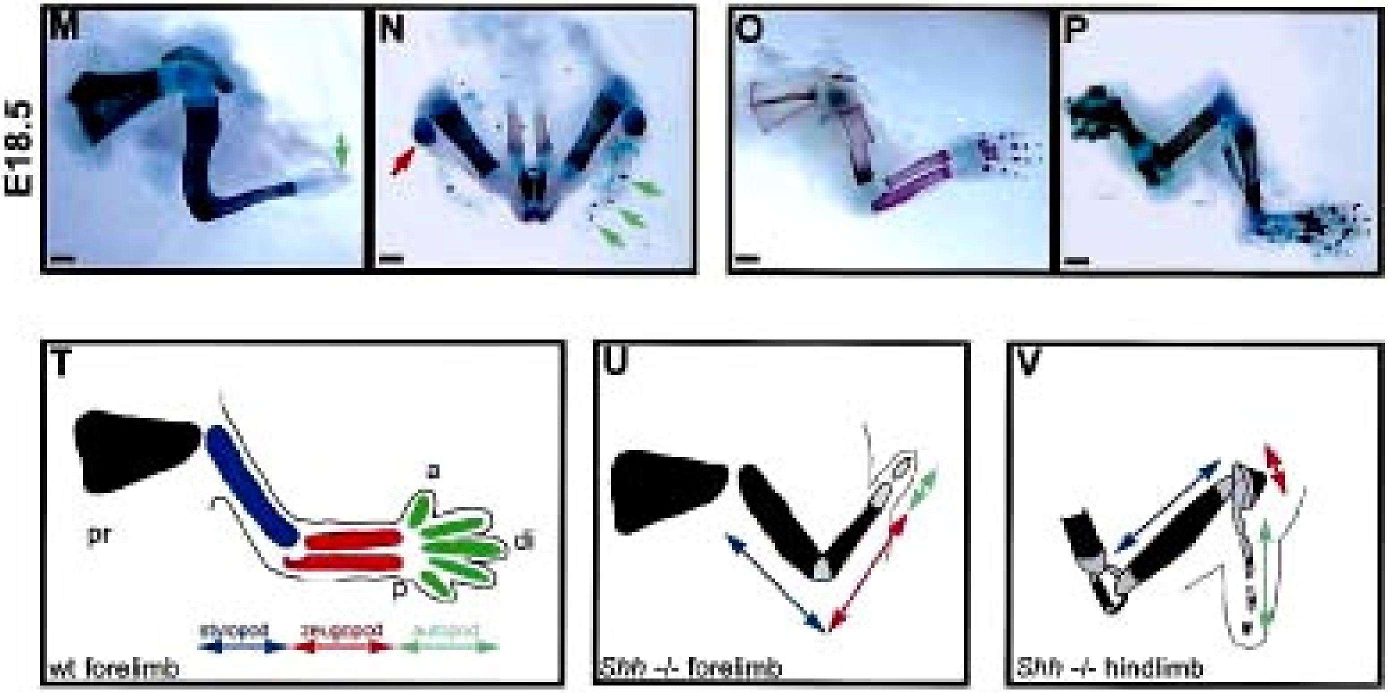
Sonic hedgehog causes ZPA-like duplications

Wild Type



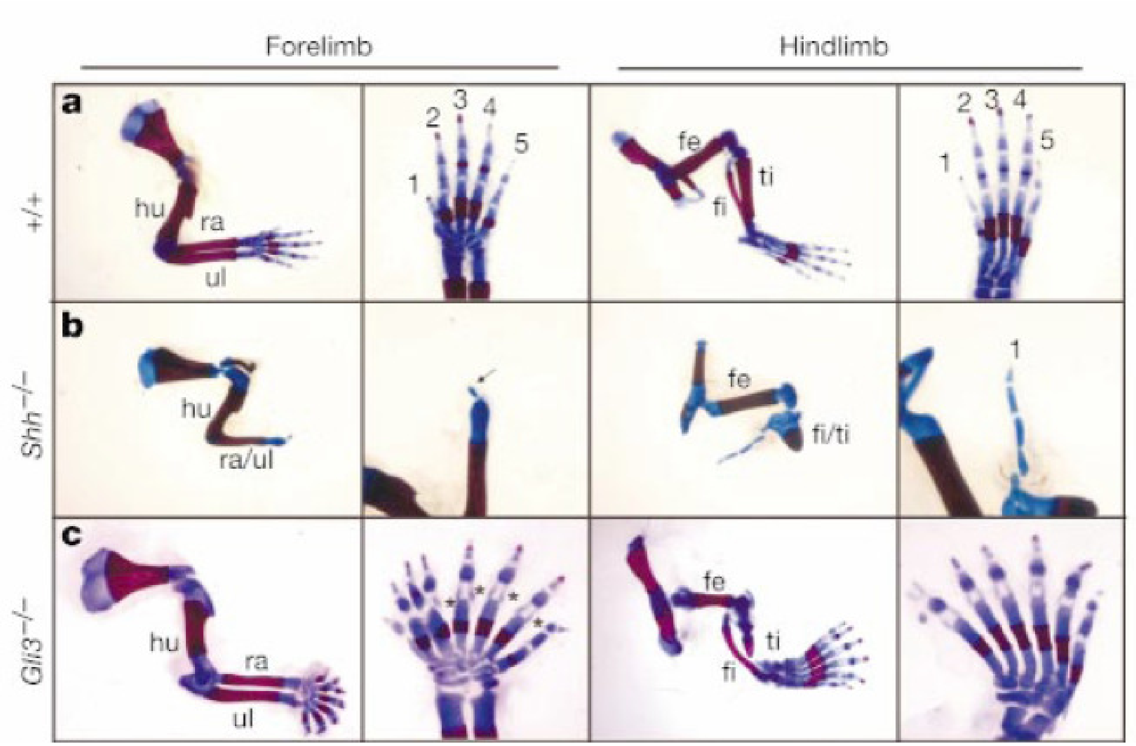
Sonic Protein Implant

The complementary approach:
Deletion or ‘knock-out’ of the *Shh* gene leads to a disruption in anterior-posterior patterning of the limb



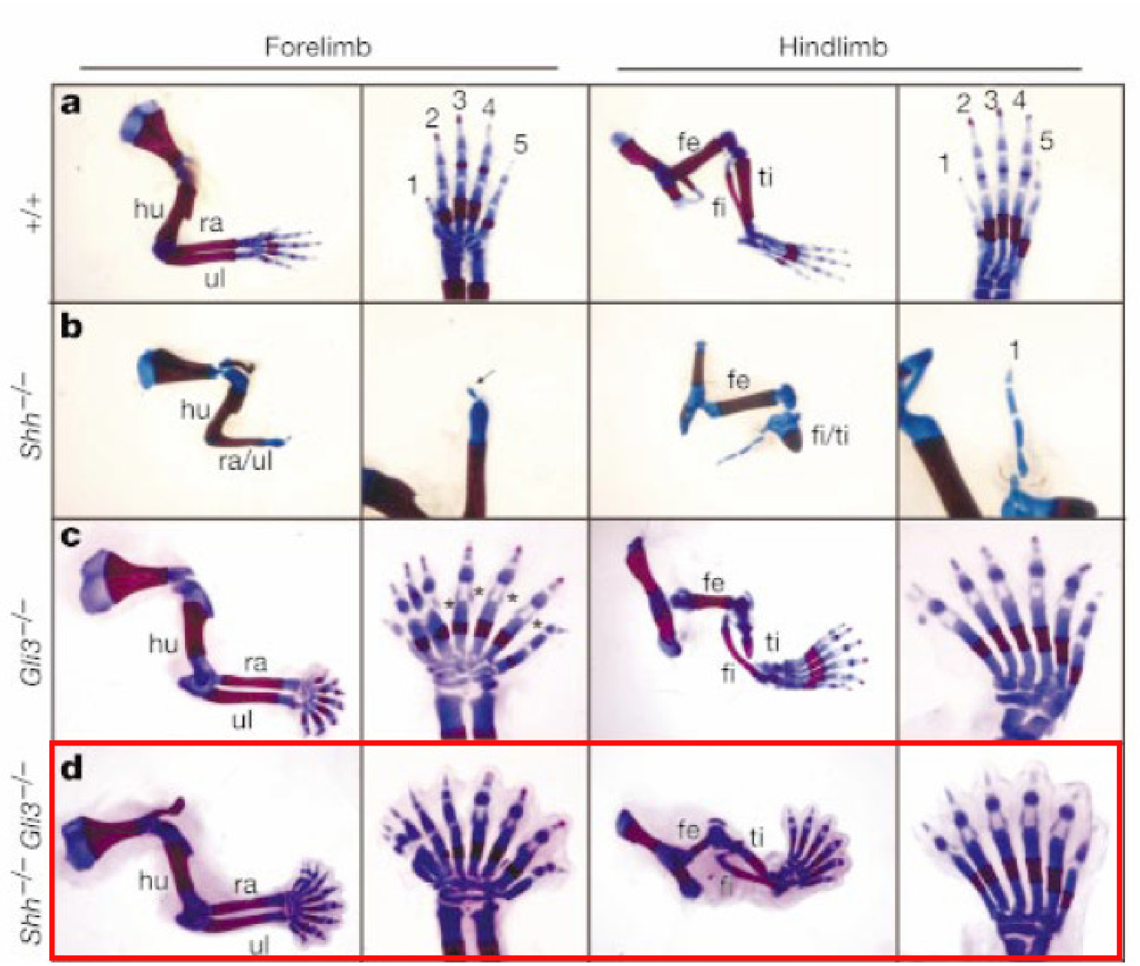
Chiang et al., Nature 1996
Kraus et al., Mech Dev. 2001

Biochemistry: Gli3 transcription factor mediates Shh function



Gli3 loss-of-function results in polydactyly.

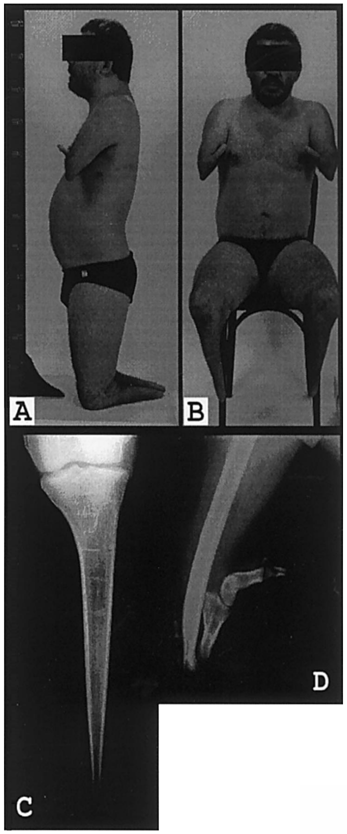
Surprisingly, *Shh/Gli3* double mutants look identical to *Gli3* nulls



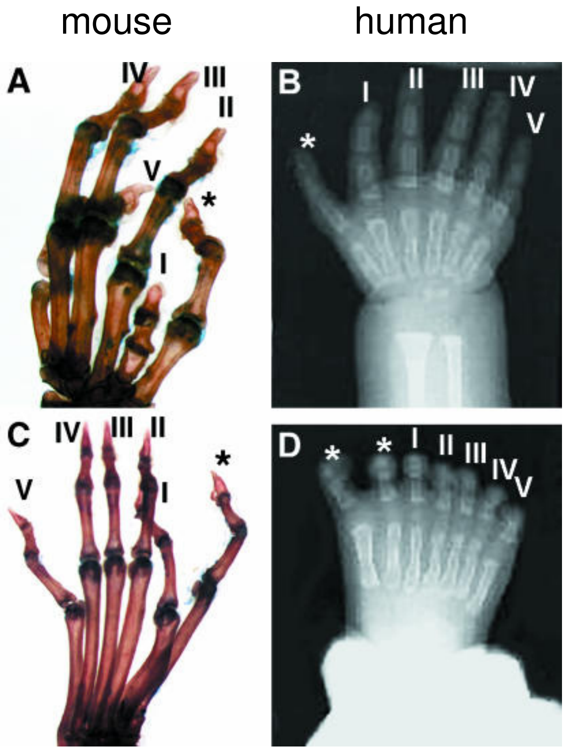
Shh modulates inherent polydactylous limb ‘ground state.’

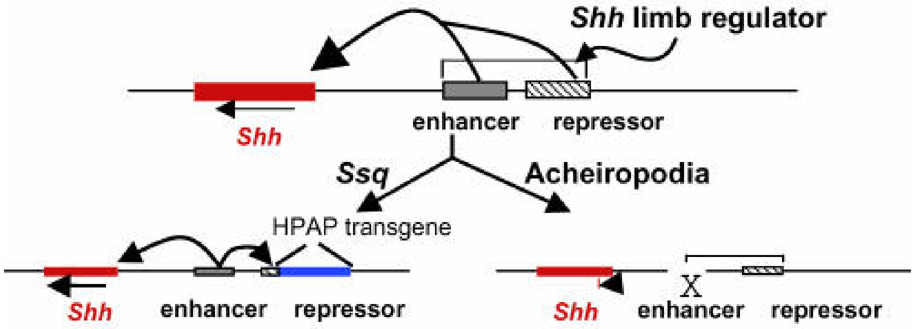
Shh inhibits an inhibitor of digit formation and imposes polarity

Acheiropodia: deletion of the SHH limb enhancer



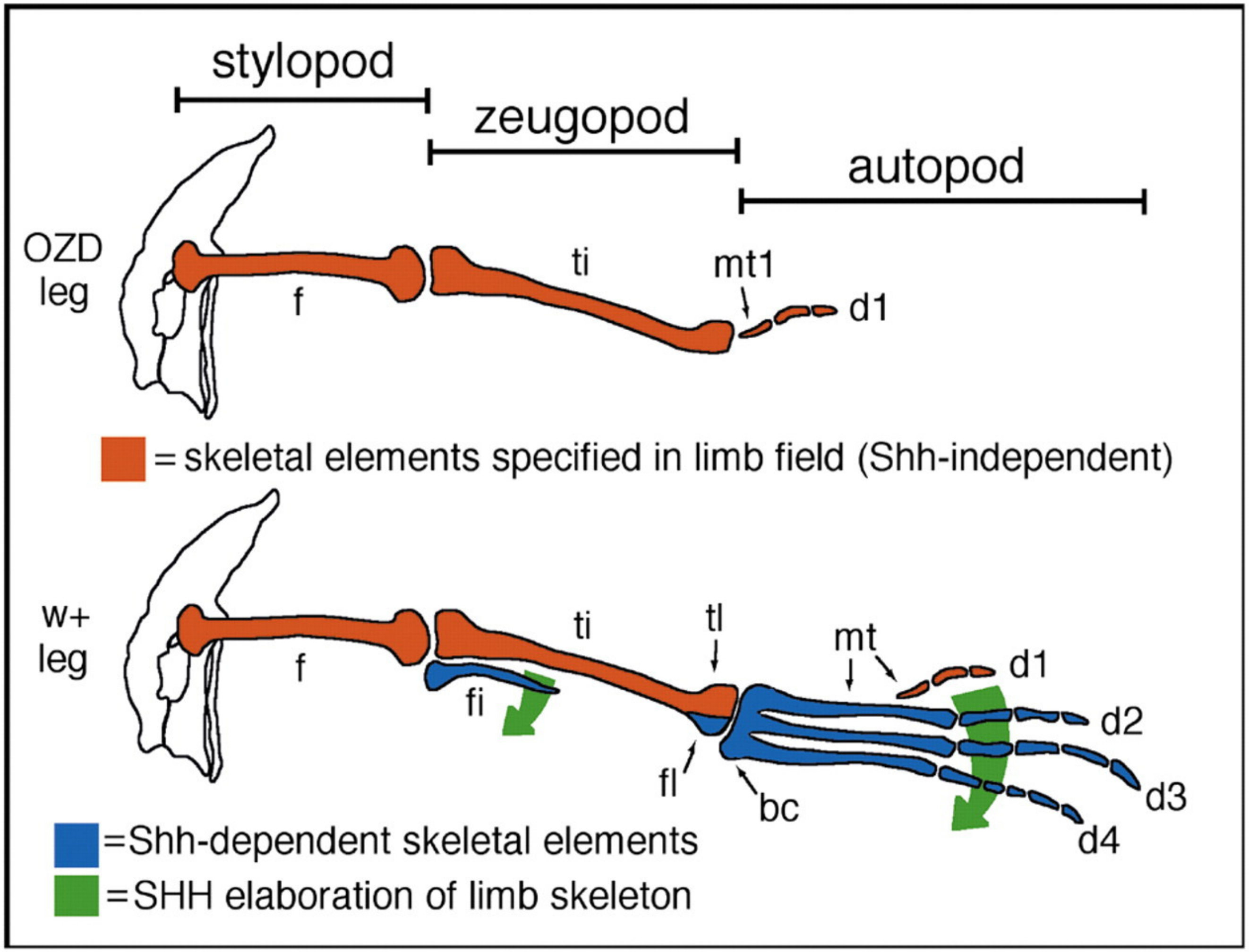
preaxial polydactyly: ectopic anterior SHH activity





J Anat. 2003 January; 202(1): 13–20.
doi: 10.1046/j.1469-7580.2003.00148.x.

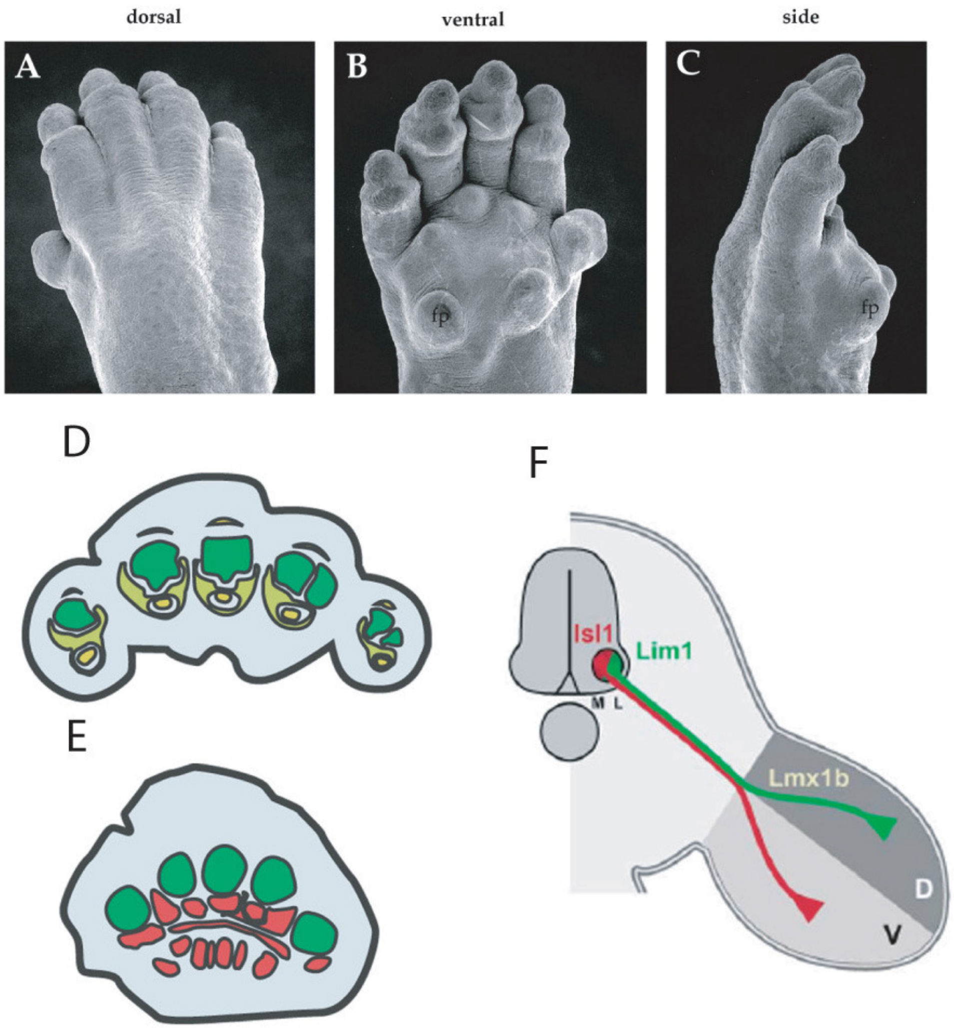
SHH function in generating the amniote limb skeleton



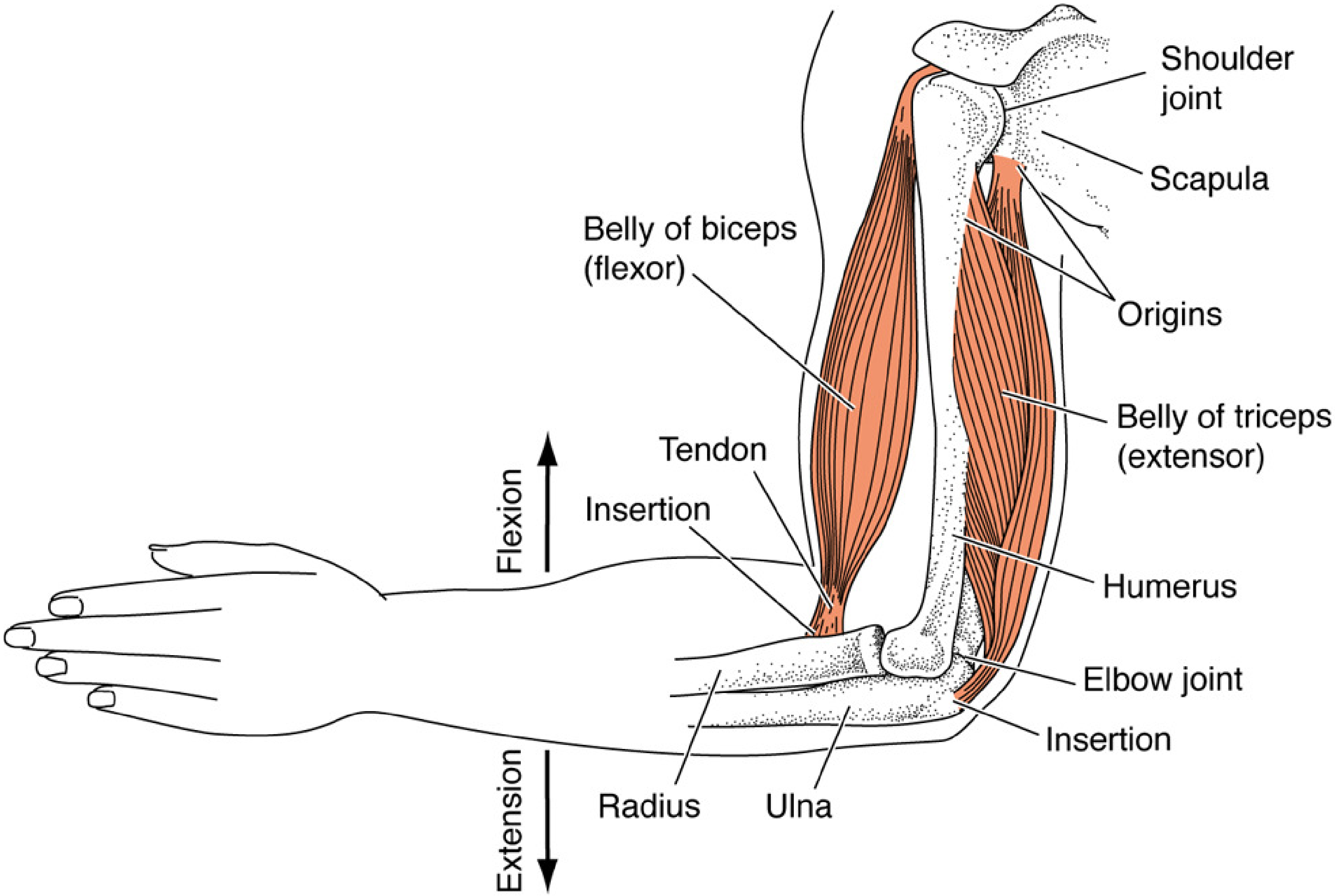
Ros, M. A. et al. Development 2003;130:527-537

The Dorsal-Ventral Axis

Dorsal-ventral limb asymmetry

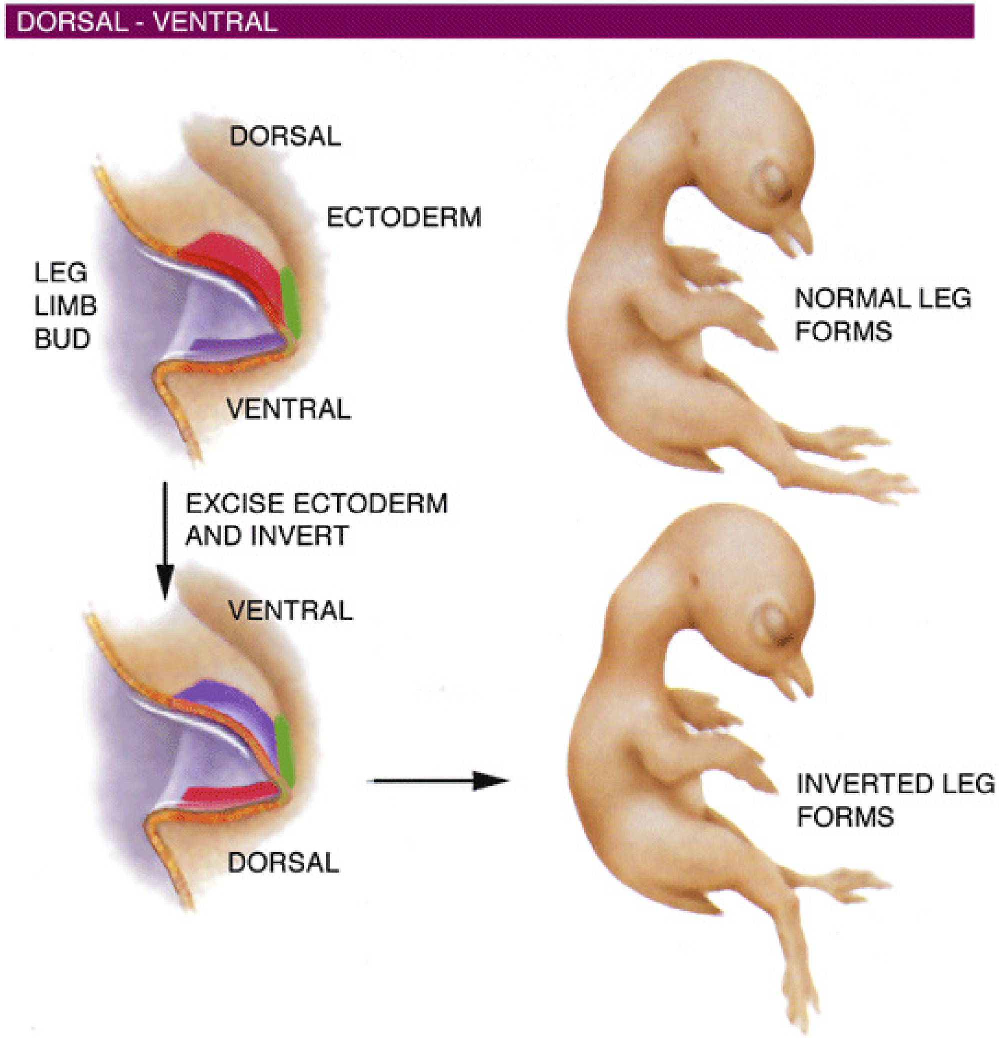


Dorsal-ventral asymmetry is required for coordinated limb movement

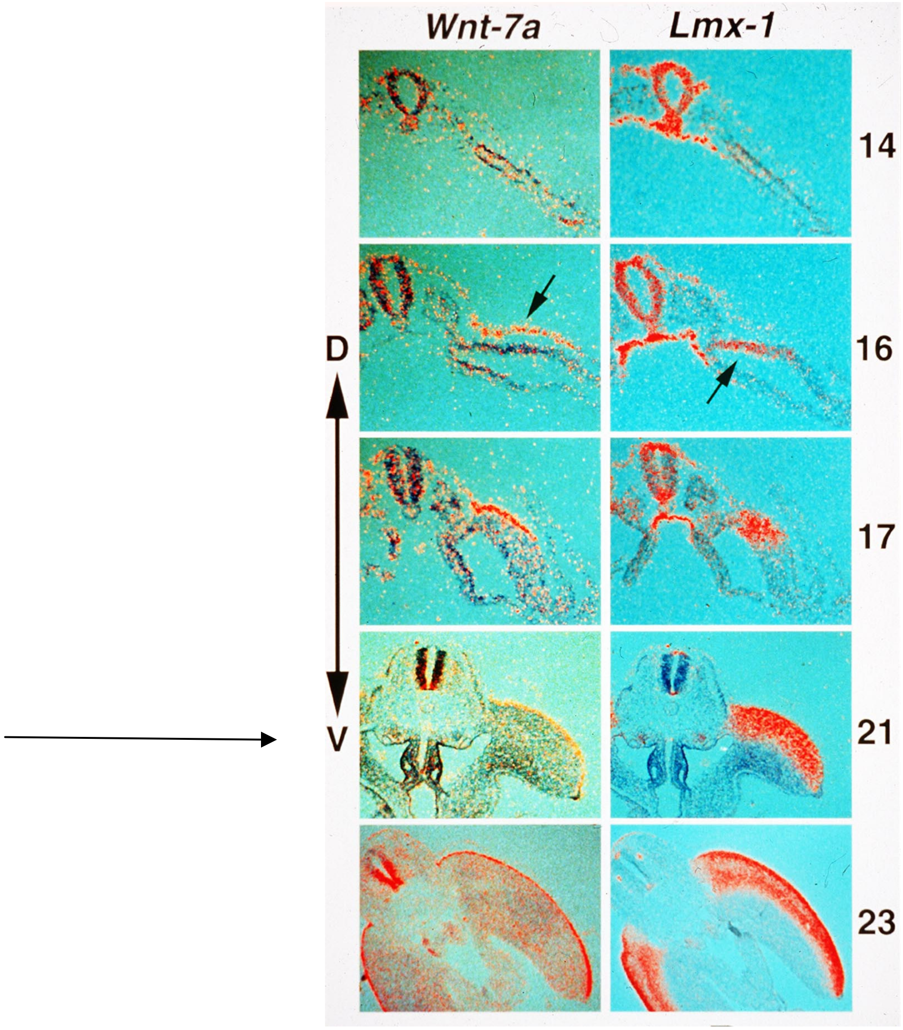


Signals from the dorsal ectoderm play an important role in patterning the dorsal-ventral axis

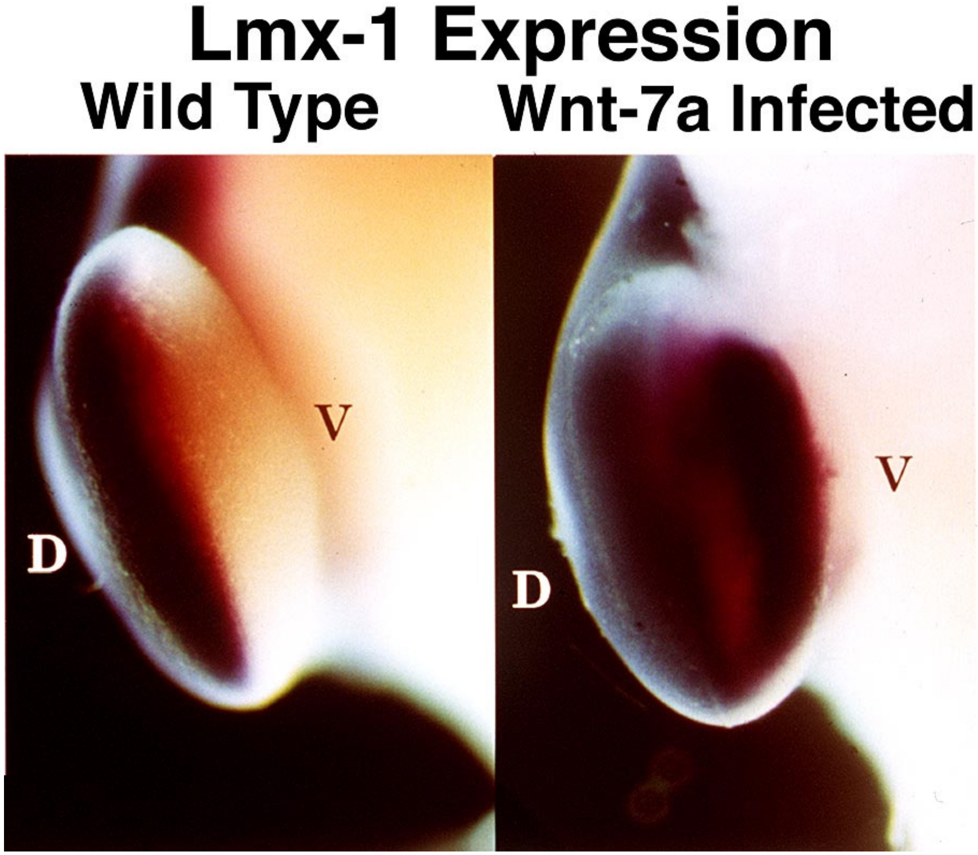
Initial observations in 'classical' embryological experiments



Candidates identified

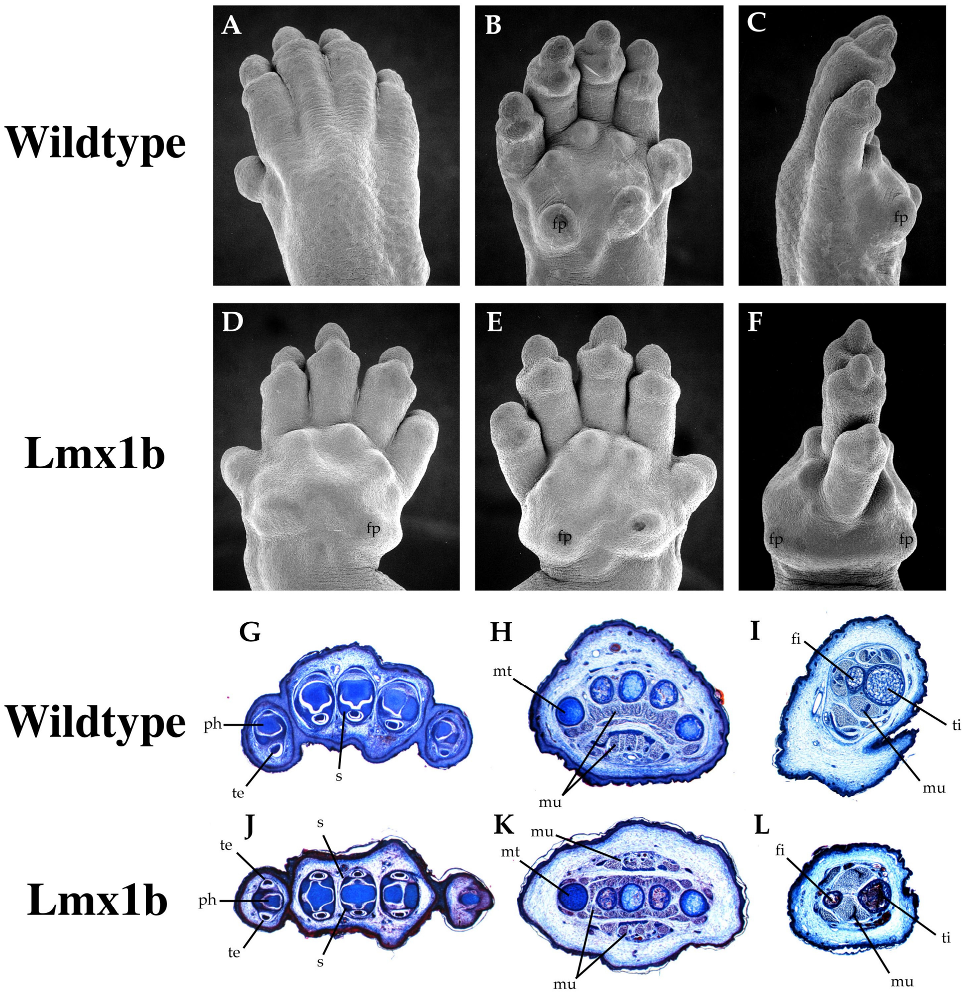


Misexpression of Wnt7a in the ventral ectoderm leads to the ectopic induction of Lmx-1 in the ventral mesenchyme

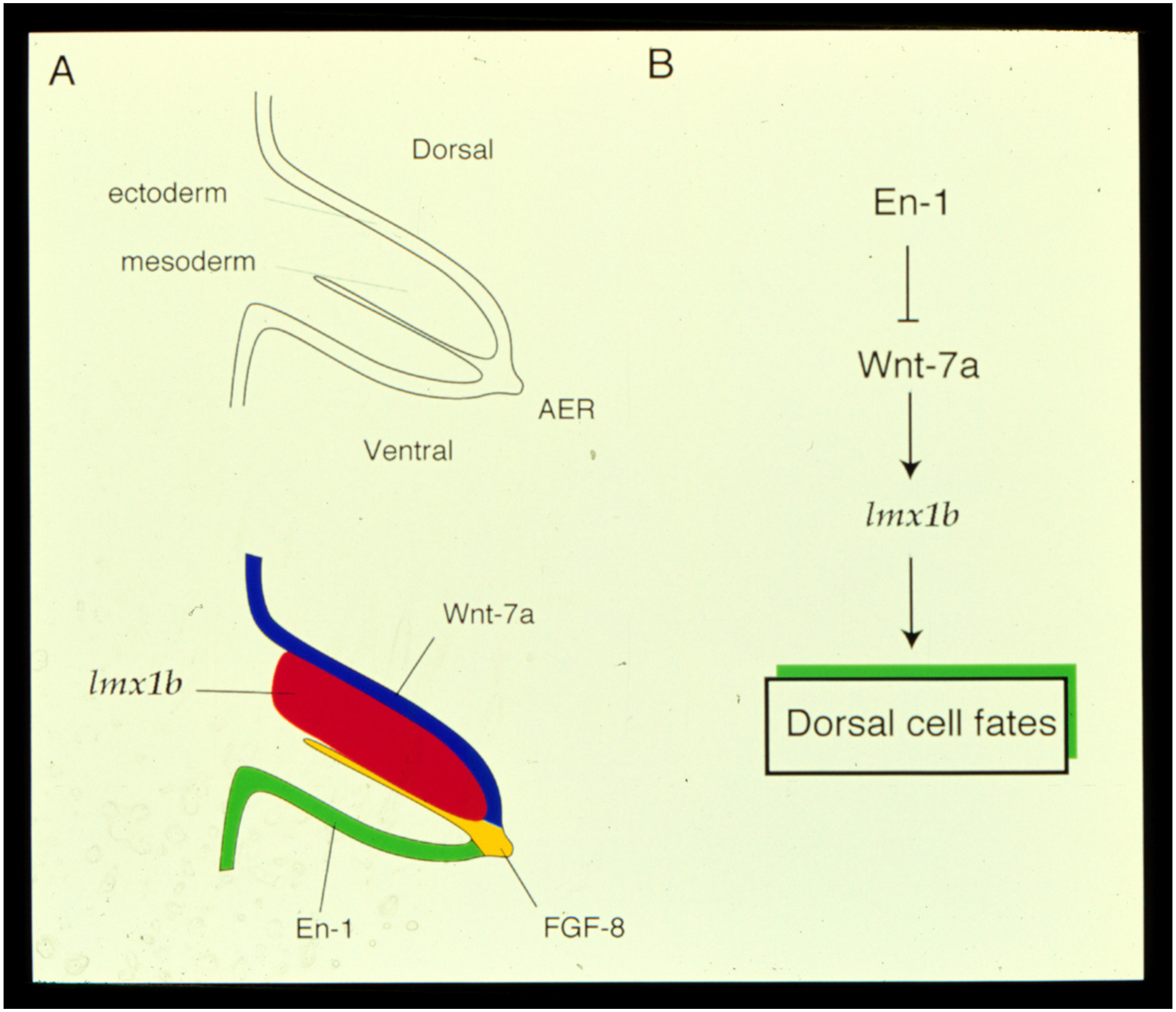


Genetic deletion of *Lmx1b* in the mouse leads to a loss of dorsal limb pattern

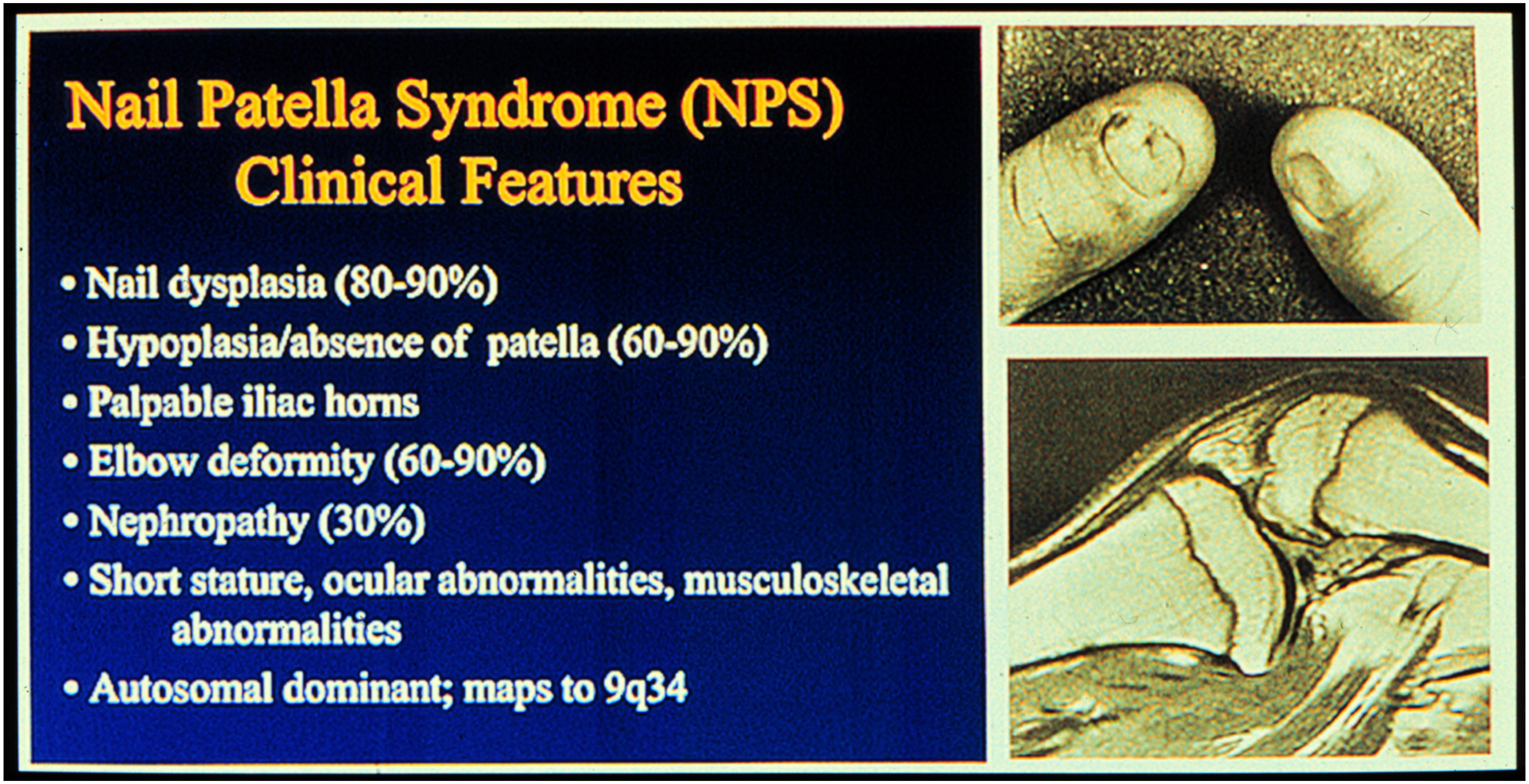
Lmx1b is required for dorsal limb patterning



A cascade of factors in the ectoderm controls dorsal-ventral polarity in the mesoderm



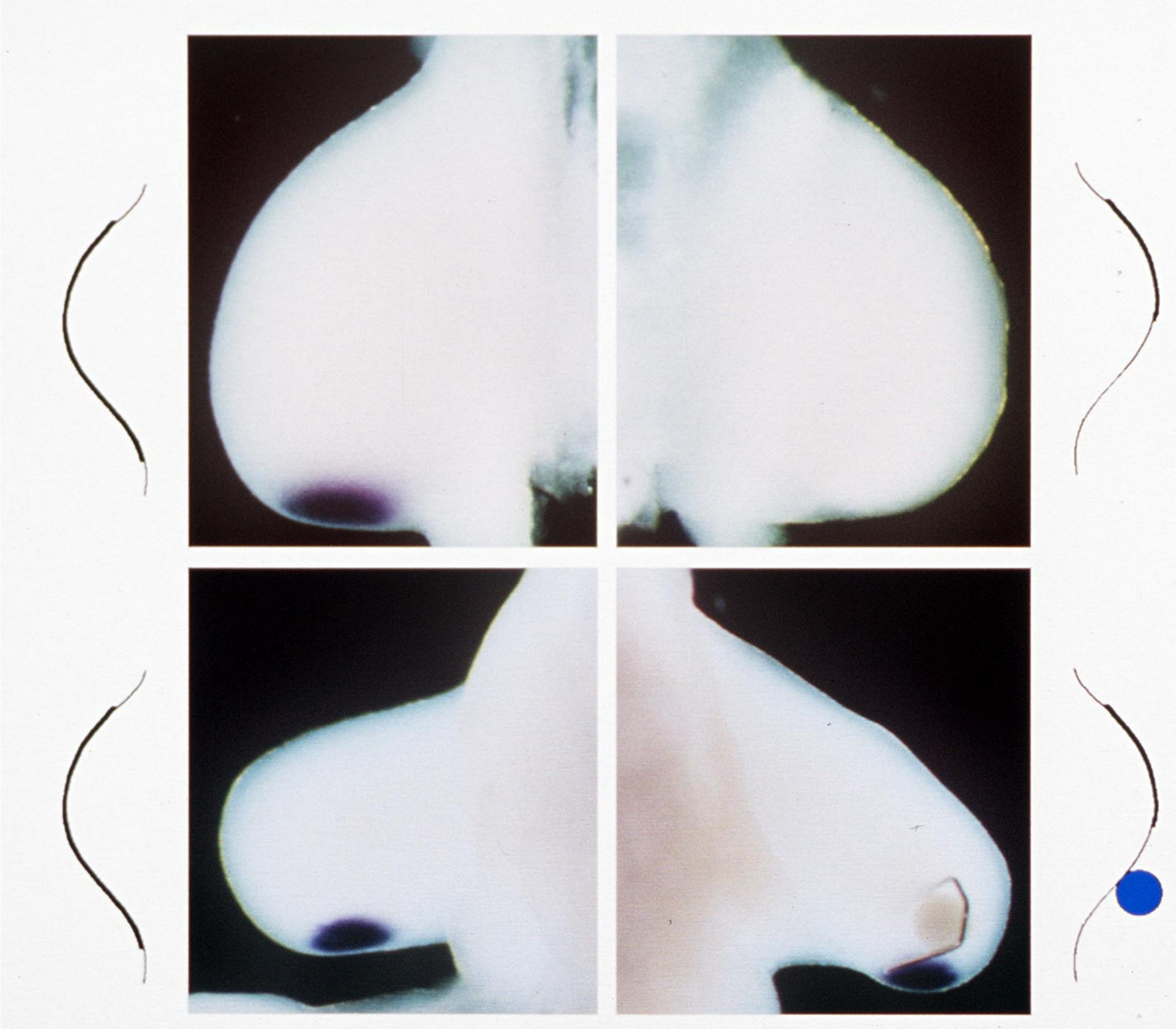
Human LMX1B mutation: Nail Patella Syndrome



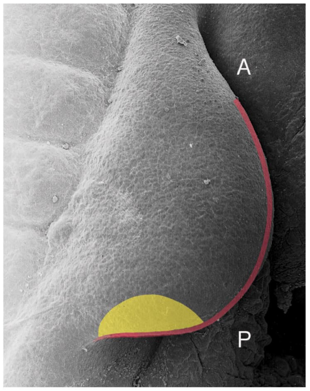
Nail Patella Syndrome (NPS)
Clinical Features

- Nail dysplasia (80-90%)
- Hypoplasia/absence of patella (60-90%)
- Palpable iliac horns
- Elbow deformity (60-90%)
- Nephropathy (30%)
- Short stature, ocular abnormalities, musculoskeletal abnormalities
- Autosomal dominant; maps to 9q34

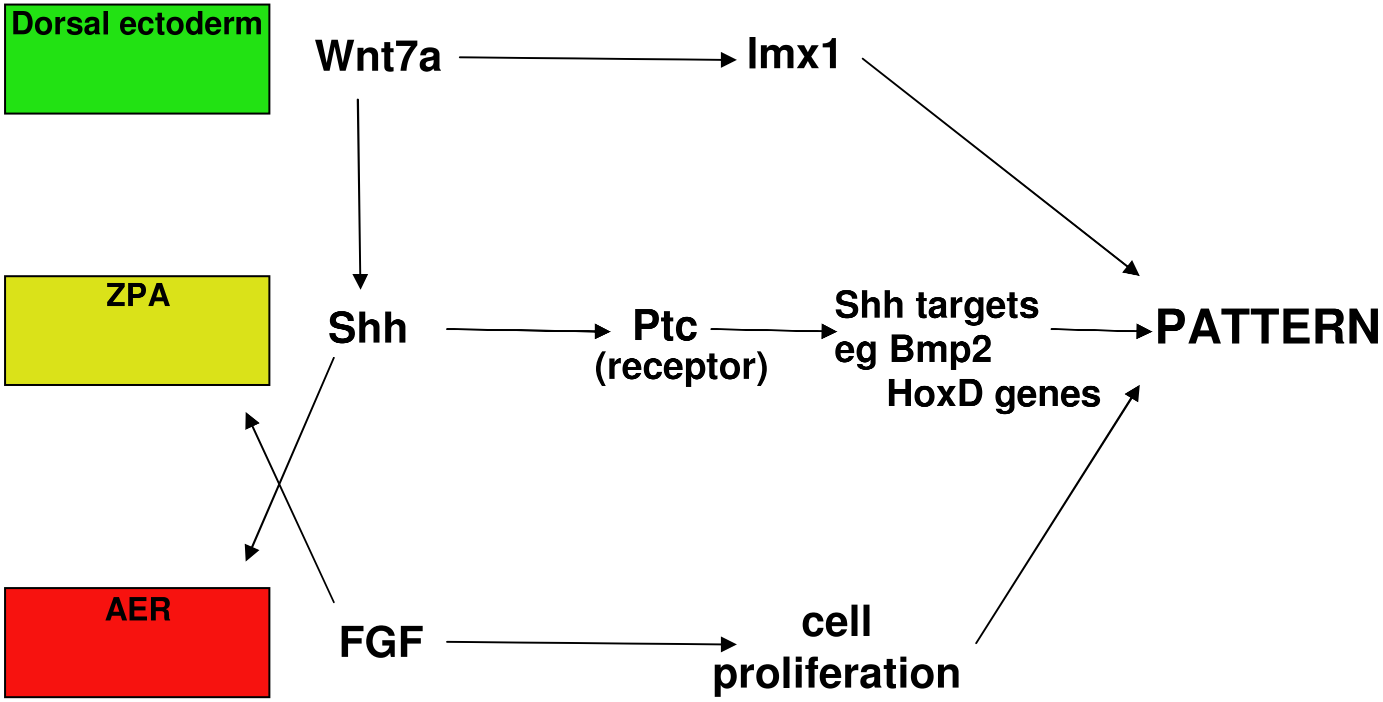
Signaling center crosstalk:
Signals from the AER (FGFs) are required to maintain expression of Shh



Limb patterning occurs through the coordination of signals from three signaling centers



Signaling centers



Dorsal ectoderm

Wnt7a → lmx1 → PATTERN

Wnt7a → Shh

ZPA

Shh → Ptc (receptor) → Shh targets eg Bmp2 HoxD genes → PATTERN

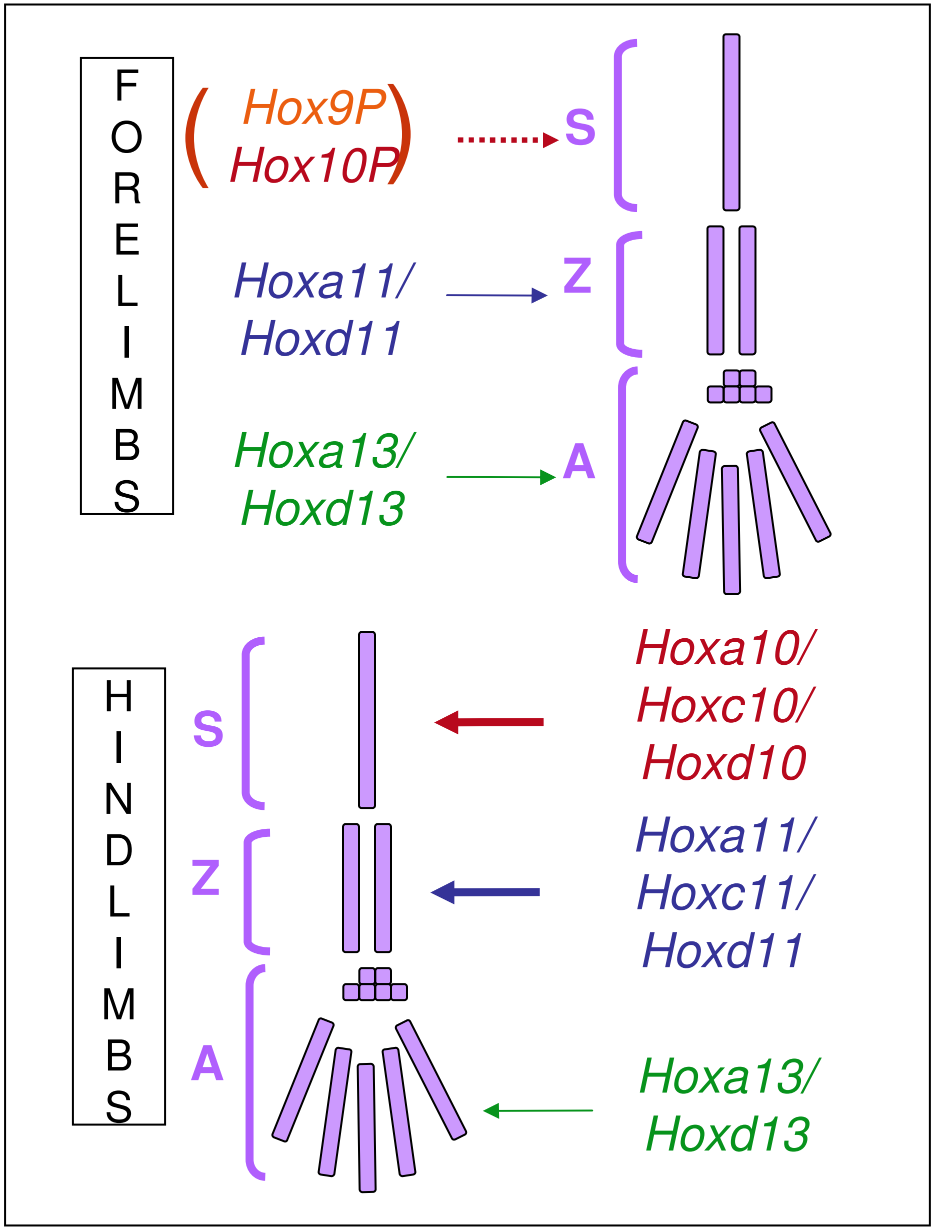
Shh → AER

AER

FGF → ZPA

FGF → cell proliferation → PATTERN

Patterning of the limb elements: *Hox9* through *Hox13* paralogous groups are responsible for establishing morphological pattern



FORELIMBS

(*Hox9P* *Hox10P*) → S

Hoxa11/ *Hoxd11* → Z

Hoxa13/ *Hoxd13* → A

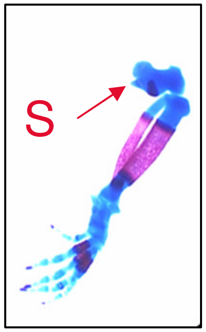
HINDLIMBS

S ← *Hoxa10*/ *Hoxc10*/ *Hoxd10*

Z ← *Hoxa11*/ *Hoxc11*/ *Hoxd11*

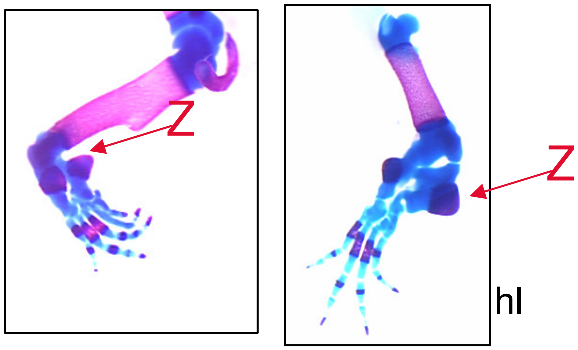
A ← *Hoxa13*/ *Hoxd13*

Hox10

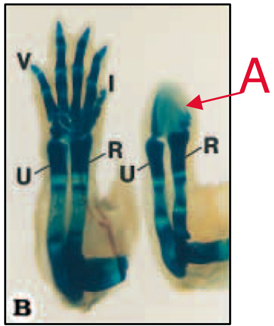


Wellik, *et al*, 2003

Hox11

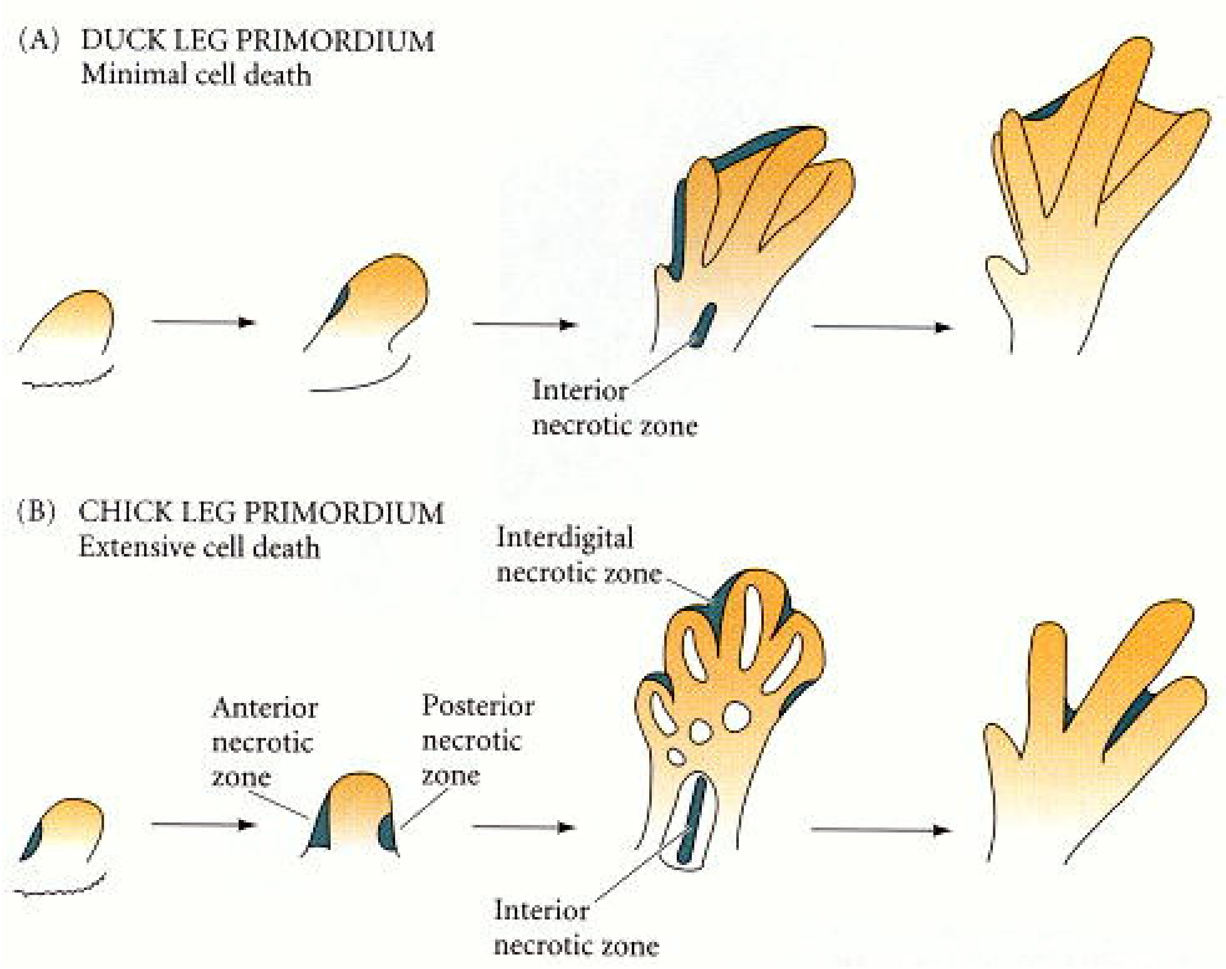


Hox13

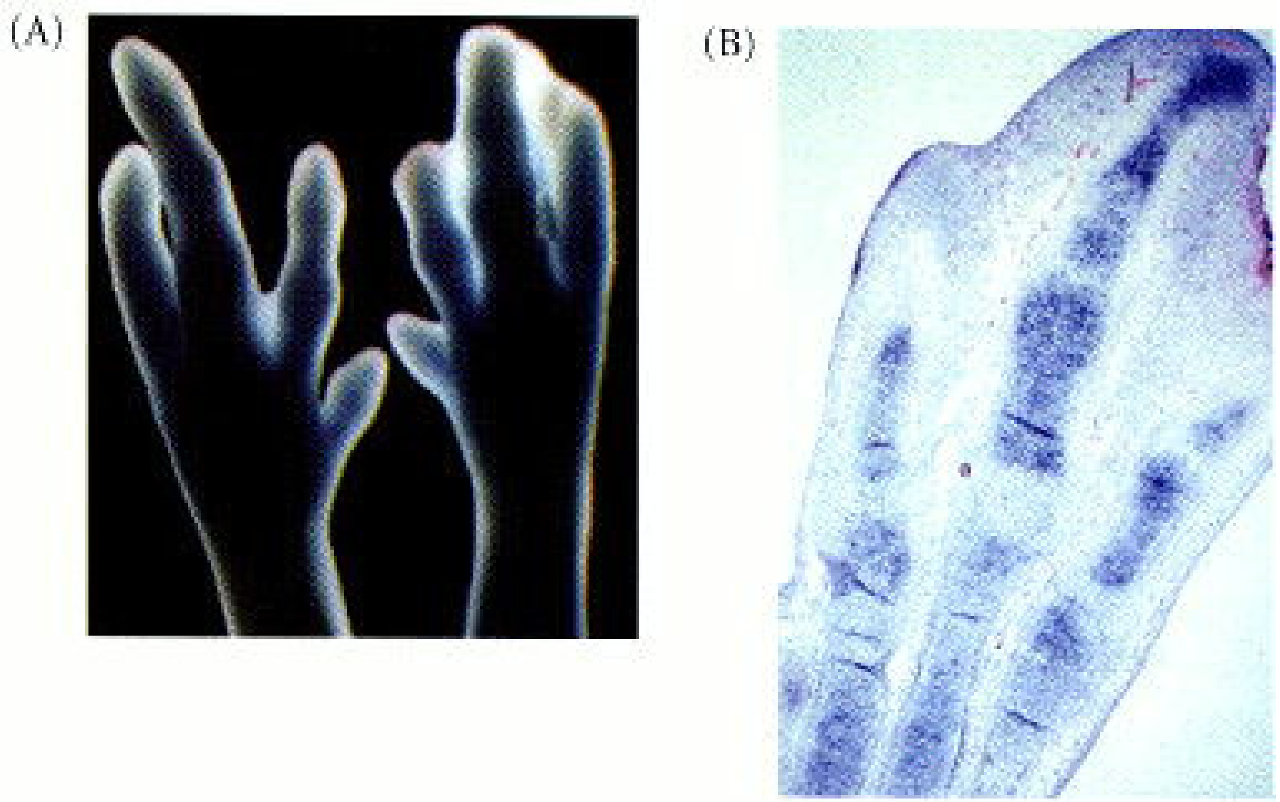


Fromental-Ramain, *et al,* 1996

Apoptosis regulates interdigit formation

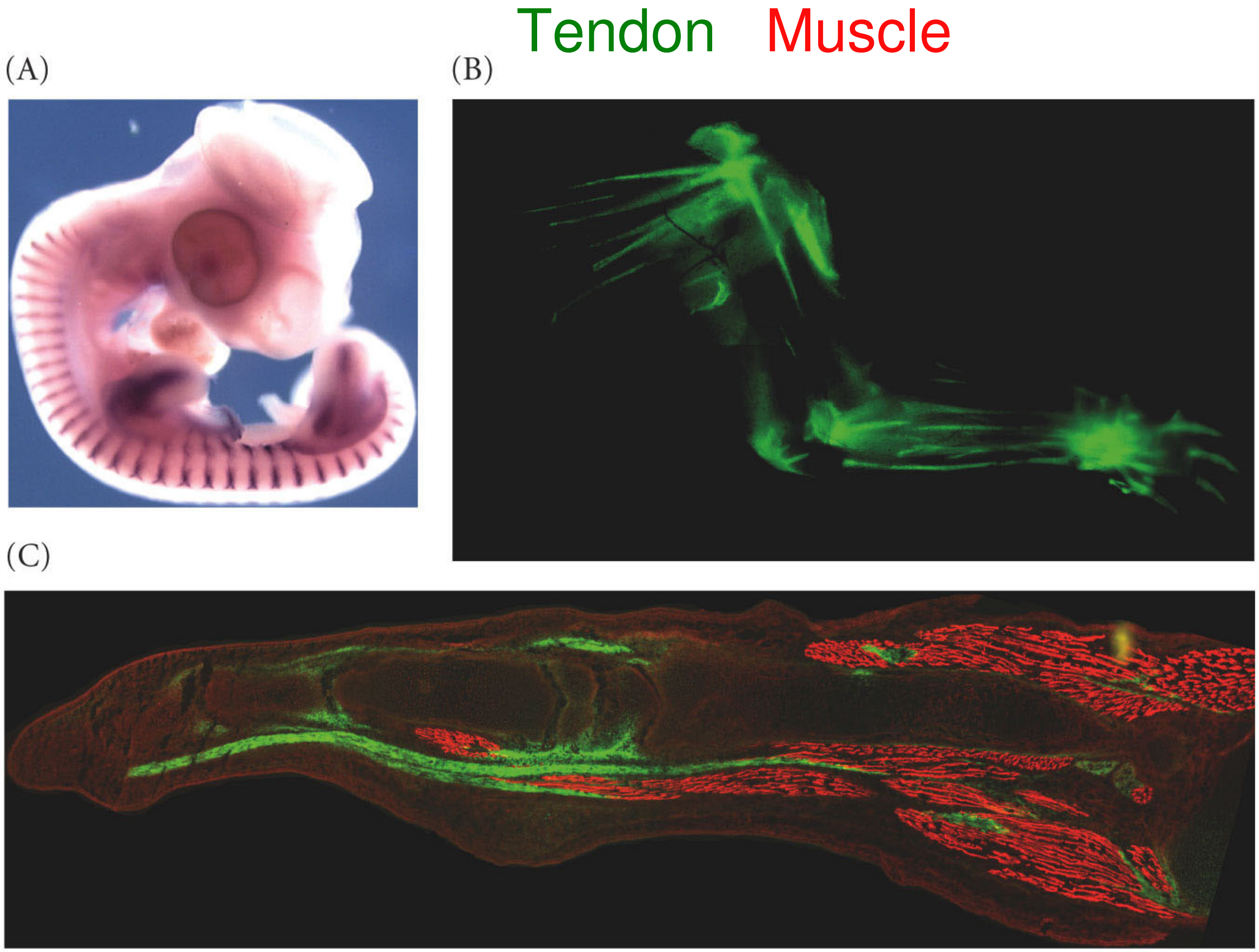


BMP signaling regulates interdigital apoptosis



Dominant-negative BMP receptors inhibit interdigit cell death

Tendons develop in situ, while muscle progenitors migrate in from the somites and follow a prepattern established in the limb mesenchyme



DEVELOPMENTAL BIOLOGY, Eighth Edition, **Figure 14.18**

Summary

- Initiation: localized FGF activity
- Outgrowth: FGFs produced by the AER
- Anterior-posterior patterning: ZPA/Sonic hedgehog
- Dorsal-ventral patterning: engrailed-wnt7a-lmx1b
- Integration of signaling centers
- Tbx genes are required for limb outgrowth
- Hox genes are essential regulators of limb development and control segmental development
- Apoptosis sculpts interdigital regions.